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TWO PARTS
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The Journal of Clinical Investigation

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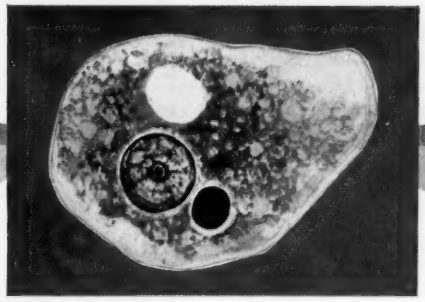

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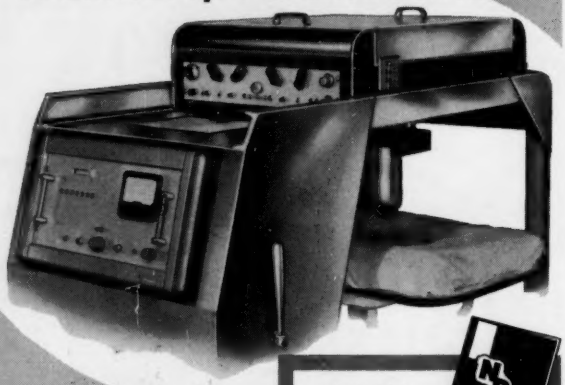

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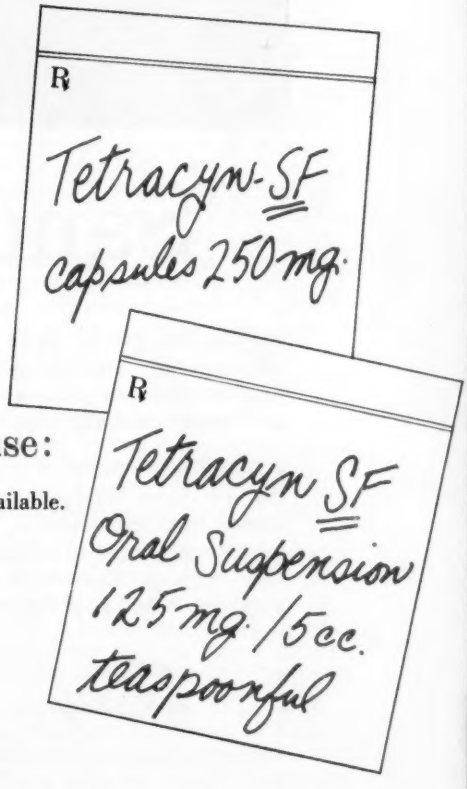

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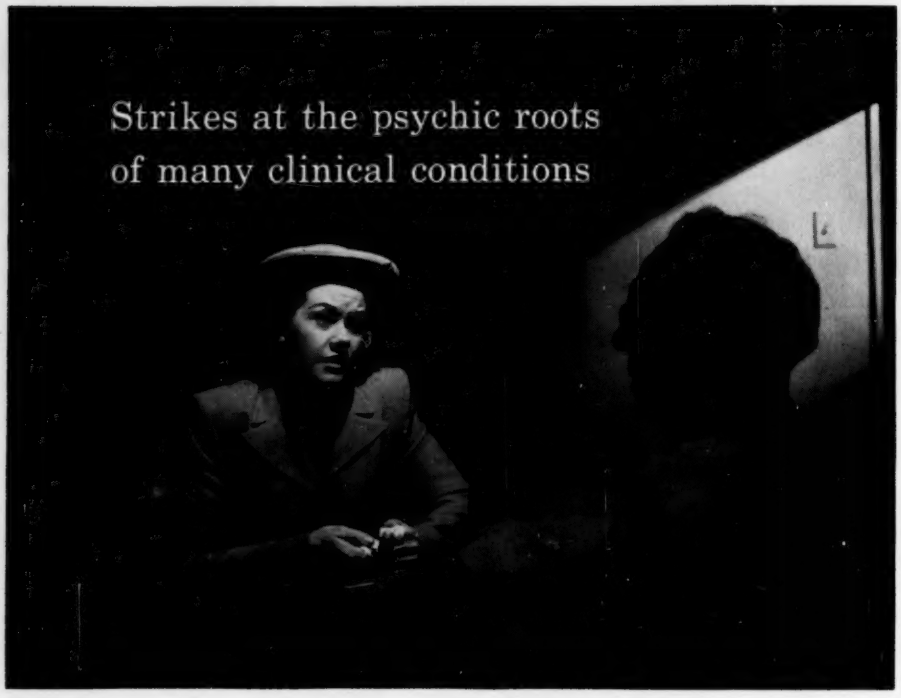

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STUDIES OF RESPIRATORY PHYSIOLOGY IN THE NEWBORN INFANT. I. OBSERVATIONS ON NORMAL PREMATURE AND FULL-TERM INFANTS [1]

By CHARLES D. COOK, RUTH B. CHERRY, DONOUGH O'BRIEN,[2] PETTER KARLBERG,[3] AND CLEMENT A. SMITH

(From the Boston Lying-in Hospital and the Department of Pediatrics, Harvard Medical School, Boston, Mass.)

(Submitted for publication January 3, 1955; accepted February 2, 1955)

This report describes an investigation of the respiratory physiology of normal premature and full-term newborn infants. As in previous studies on normals (1–3), minute volumes, rates and tidal volumes have been measured. Additional measurements of CO_2 production, plasma CO_2 partial pressure, and intraesophageal pressure differences have allowed calculations of effective alveolar ventilation, functional dead space and estimates of the work of respiration. Since certain adaptations were necessary for the study of this age group, the techniques are reported in detail. The data from normal infants are the subject of this report; comparable data for newborns with respiratory distress are reported in a second paper (4).

MATERIAL

The 63 infants studied by one or more techniques were delivered at the Boston Lying-in Hospital and were considered normal in all respects except that 17 were premature by weight. They ranged from 36 to 42 weeks gestational age and from 1.82 to 4.25 Kg. birth weight. Delivery was spontaneous vertex or low forceps except in five infants delivered by Caesarian section (three because of previous section, one for marginal placental separation, and one for postmaturity), and four by breech extraction. Their ages at the time of observation ranged from three hours to seven days (Tables I–III). Most of those under two days had not been fed prior to the studies.

METHODS

The minute volume ($\dot{V}$),[4] rate (f), and tidal volume (V_T), were measured in 35 infants by means of a 65-liter body plethysmograph in which the infant lay on its back, with the face emerging through a pneumatic ring (Figure 1). The apparatus, which was similar to that described by Cross (2), was largely assembled by Dr. Frank Hytten. Most of these infants, none of whom were sedated, were sufficiently comfortable to sleep throughout the observations. Only data on infants who were considered to be resting and basal are included in this report. An average of seven minutes of quiet breathing was used in calculating the results.

The body plethysmograph was connected to a Krogh spirometer, and the entire system was checked for leaks with a 6 gm. weight placed on the spirometer float; maintenance of air in the spirometer for more than twenty seconds indicated an error of less than 2 per cent in minute volume recording and was considered satisfactory. Kymograph tracings of respiratory pattern were obtained from a writing pen attached to the spirometer float, and minute volume from an automatic integrator as designed by Roberts and Widdas (6). This recorder utilized a photoelectric cell and integrator to count flickers of light produced by the spirometer moving one of a pair of transilluminated grids. The entire plethysmograph-spirometer-integrator system was calibrated at least once with each study with a syringe and pump which produced rhythmical volume changes of a known amount. The calibration per ml. was constant with pump rates varying between 30 and 60 per minute. With higher rates, however, there was a progressive systematic overswing of the spirometer so that integrator counts increased per ml. When studying infants with elevated respiratory rates (only those with respiratory difficulty) a factor was applied to correct for this systematic error. At rates of 80 the correction amounted to 6 per cent and at 100 to 15 per cent.

The CO_2 production ($\dot{V}_{CO_2}$) and O_2 consumption ($\dot{V}_{O_2}$) of 16 infants were determined by using a plastic face mask with a volume of approximately 60 ml. sealed to the pneumatic ring (Figure 1) with an aquaresin-glycerine mixture.[5] Compressed air or other oxygen-nitrogen mixtures were drawn by a pump across the infant's face

[1] One of a series of studies supported by a research grant from the Association for the Aid of Crippled Children, New York City.

[2] Work done under Grocers' Company Research Scholarship, and Research Fellowship from the Lederle Company; Present Address: Department of Child Health, Guy's Hospital, London.

[3] Work done under Rockefeller Fellowship; Present Address: Karolinska Sjukhuset, Stockholm.

[4] Throughout this paper the symbols employed to denote respiratory variables are those suggested for purposes of standardization by a group of clinical and research respiratory physiologists headed by J. R. Pappenheimer (5).

[5] Aquaresin, obtained from the Glyco Products Co., Brooklyn, N. Y., was mixed with equal parts glycerine. This provided an effective seal without injuring the rubber cuff.

TABLE I

Minute volume, respiratory rate and tidal volume of 35 normal infants *
(All volumes are BTPS)

Infant No.	Birth weight Kg.	Age	Weight† Kg.	Inspired‡ gas	Min.§	$\dot{V}$ ml.	f	V_T ml.
6	2.41	3 d.	2.42	R.A.	8.0	454	27	17
7	2.71	6 hr.	—	R.A.	6.5	473	36	13
10	4.08	7 hr.	—	R.A.	8.0	770	37	21
11	2.51	6 hr.	—	R.A.	11.0	330	30	11
12	2.67	30 hr.	2.68	R.A.	4.0	552	34	16
13	2.33	5 hr.	2.31	R.A.	33.0	520	34	15
14	2.75	5 hr.	—	R.A.	7.0	515	27	19
17	2.06	2 d.	1.88	R.A.	7.0	290	46	6
25	3.06	11 hr.	—	R.A.	8.0	485	27	18
26	2.62	12 hr.	—	R.A.	4.5	584	46	13
30	2.41	4 d.	2.38	R.A.	9.5	405	31	13
32	3.16	12 hr.	2.86	R.A.	3.0	569	33	17
37	3.02	29 hr.	2.85	C.A.	5.0	458	31	15
38	2.50	4 d.	2.28	R.A.	3.0	420	32	13
45	2.60	24 hr.	2.42	R.A.	2.5	463	40	12
48	2.33	12 hr.	2.25	C.A.	2.0	538	35	15
51	2.98	5 hr.	2.85	C.A.	5.5	486	38	13
56	3.31	7 d.	3.06	C.A.	6.0	840	40	21
57	3.23	3 d.	2.93	C.A.	3.5	564	28	20
58	2.55	10 hr.	2.50	C.A.	3.0	402	33	12
60	2.88	20 hr.	2.90	C.A.	8.5	746	51	15
61	2.50	13 hr.	2.49	C.A.	2.0	845	49	17
62	2.36	16 hr.	2.29	C.A.	4.5	421	23	18
63	2.72	21 hr.	2.65	C.A.	7.5	466	32	15
65	3.04	25 hr.	2.82	C.A.	2.5	550	28	20
66	3.92	16 hr.	3.75	C.A.	7.0	579	30	19
68	3.72	17 hr.	3.61	C.A.	4.0	579	28	21
69	1.82	21 hr.	1.76	C.A.	2.0	383	26	15
71	3.52	21 hr.	3.32	C.A.	17.0	481	29	17
76	2.64	11 hr.	2.63	C.A.	10.0	600	29	21
80	2.46	15 hr.	2.35	C.A.	4.0	435	31	14
81	2.81	12 hr.	2.58	C.A.	8.5	453	33	14
83	3.26	23 hr.	3.09	C.A.	13.5	564	40	14
84	1.94	16 hr.	—	C.A.	5.0	480	37	13
86	2.28	2 d.	2.04	C.A.	6.0	444	32	14

* BTPS—Body temperature, pressure, saturated with water vapor.
$\dot{V}$—minute volume.
f—respiratory rate per minute.
V_T—tidal volume.

† Seven infants studied during the first 24 hours after birth were not weighed at time of study; their birth weights are used in Figure 2.

‡ R.A. = room air.
C.A. = compressed air.

§ Number of resting minutes averaged.

at the rate of 5 to 6 liters per minute and delivered, together with the expired air, into plastic collecting bags. At this rate, analyses of mask air never showed more than 0.3 volumes per cent CO_2. A small series of studies on the same infants breathing room air and then compressed air, and a comparison of the group of infants studied in room air with the group studied in compressed air showed no statistically significant difference in minute volume ($P = 0.95$). Thus it seems probable that respiration was not appreciably influenced by the use of the mask. Gas collection periods used for calculations averaged 3.5 minutes; the volume collected was measured with a dry gas meter which had an error of 1.5 per cent or less.

Analyses for CO_2 and O_2 in the inspired gas and in that from the collecting bags were performed in the Scholander 0.5 ml. gas analyzer (7). Analyses of CO_2 were made at least in duplicate in every case, with a standard deviation from the averages of these duplicate determinations of ± 0.007 volumes per cent for the inspired air, and ± 0.013 volumes per cent for the diluted expired air. This degree of reproducibility amounted to a variation of approximately ± 5 per cent of the total CO_2 production per minute. Oxygen analyses, also performed at least in duplicate, showed a standard deviation from the averages of ± 0.012 volumes per cent for both inspired and expired air. The Scholander analyzer was checked against a Haldane apparatus with resultant

TABLE II

Additional physiologic observations in 18 normal infants *
(All volumes are BTPS)

Infant No.	Birth weight Kg.	Age	$\dot{V}$ ml.	f	V_T ml.	$\dot{V}_{CO_2}$ ml.	$\dot{V}_{O_2}$ ml.	R	Ca_{CO_2} vol. %	pH	Pa_{CO_2} mm. Hg	$\dot{V}_A$ ml.	V_D ml.	V_D/V_T
48	2.33	12½ hr.	600	36	17	17.5	21.6	.81	—	—	—	—	—	—
51	2.98	5 hr.	498	39	13	15.5	21.2	.73	—	—	—	—	—	—
56	3.31	7 d.	825	39	21	25.9	36.0	.72	47	7.40	32	581	6.3	.30
58	2.55	10 hr.	418	33	13	12.9	19.8	.65	51	7.37	37	241	5.4	.42
60	2.88	20 hr.	751	52	14	16.0	22.8	.70	43	7.35	33	351	7.7	.55
61	2.50	13 hr.	835	50	17	16.4	20.2	.81	39	7.41	26	452	7.7	.45
62	2.36	16 hr.	465	24	19	14.4	24.4	.59	44	7.42	29	354	4.6	.24
63	2.72	21 hr.	486	34	14	12.6	20.3	.62	49	7.40	34	272	6.3	.45
65	3.04	25 hr.	562	29	19	16.4	19.9	.82	54	7.43	35	344	7.5	.39
66	3.92	16 hr.	578	29	20	18.2	25.5	.71	57	7.45	35	370	7.2	.36
68	3.72	17 hr.	590	28	21	19.5	28.1	.69	49	7.43	31	446	5.1	.24
69	1.82	21 hr.	383	26	15	10.5	22.0	.48	46	7.51	25	302	3.1	.21
71	3.52	21 hr.	463	28	17	15.0	21.0	.71	49	7.50	27	398	2.3	.14
76	2.64	11 hr.	676	31	22	22.2	24.7	.90	54	7.46	32	492	5.9	.27
80	2.46	15 hr.	435	31	14	13.3	18.4	.72	53	7.35	40	234	6.5	.46
83	3.26	23 hr.	543	39	14	17.5	22.2	.79	51	7.47	30	410	3.4	.24
84	1.94	16 hr.	480	37	13	14.7	17.4	.84	42	7.45	26	418	1.7	.13
86	2.28	2 d.	444	32	14	11.5	14.8	.78	51	7.52	27	307	4.3	.31

* $\dot{V}_{CO_2}$—CO_2 production per minute.
$\dot{V}_{O_2}$—O_2 consumption per minute.
R—respiratory exchange ratio.
Ca_{CO_2}—CO_2 concentration in plasma.
Pa_{CO_2}—CO_2 pressure in plasma.
$\dot{V}_A$—alveolar ventilation per minute.
V_D—functional dead space.

All infants were breathing compressed air; the $\dot{V}$, f, and V_T values differ from those given in Table I for the same infants, as they were obtained during observation periods necessarily shortened for measurement of CO_2 production and O_2 consumption.

agreement within 3 per cent of the V_{CO_2} measured and 6 per cent of the V_{O_2}.

"Arterialized" capillary blood for pH and CO_2 analyses was obtained by heating the foot for ten minutes in 45 to 47°C. water, puncturing the heel, and drawing the freely flowing blood into a funnel-syringe combination with heparin [6] and mineral oil filling the dead space and mercury present for mixing (8). The effect of this brief air exposure upon blood pH and CO_2 was repeatedly demonstrated to be less than the small error of the analysis. Moreover, the pH and CO_2 values obtained were similar to those reported by Graham and his co-workers for temporal artery blood of normal infants (9). The blood samples were taken immediately after the period of gas collection and without changing the inspired gas mixture. Although the infants often cried briefly when the heel was punctured, a comparison between analyses on the first few drops of blood and later drops showed no significant change. In several infants, repeated blood samples obtained two to three times during periods up to two hours showed no significant change in pH and CO_2 concentration (Ca_{CO_2}); therefore the post-observation blood value was assumed to be representative of the entire collection period.

Plasma pH, as determined in duplicate or triplicate with a Beckman glass micro-electrode adapted to a Cambridge pH meter (10) and reproducible within ± 0.01 pH units, was corrected for temperature as suggested by Rosenthal (11). Plasma CO_2 content was directly determined in duplicate by the micro-Scholander syringe technique (12) with a standard deviation of ± 0.9 volumes per cent.

Intraesophageal pressure changes, which have been shown to be an index of intrapleural pressure differences (13), were measured as described by McIlroy, Marshall, and Christie (14) and Mead, McIlroy, Selverstone, and Kriete (15) during the quiet respiration of 28 infants. A 1 mm. internal diameter polyethylene catheter with multiple openings near the distal end was filled with water, attached to an electric manometer-amplifier-recording [7] system, and passed approximately 10 cm. through the nose or mouth. The system was calibrated with a water manometer after each study. The differences between maximal and minimal pressures occurring with each respiratory cycle during periods of quiet breathing were averaged for at least 15 individual breaths.

CALCULATIONS

The Henderson-Hasselbalch equation (16) was used for calculation of arterial P_{CO_2} (Pa_{CO_2}) from plasma pH and CO_2 concentration. The relative constancy of Pa_{CO_2} in blood drawn several times during a period of one to two

[6] The liquid heparin diluted the blood sample 0.4 per cent or less.

[7] The electric manometer, amplifier and recorder were made by the Sanborn Company, Cambridge, Massachusetts.

TABLE III

Intraesophageal pressure differences in 25 normal newborn infants

Infant No.	Birth weight Kg.	Age	ΔIEP cm. H_2O Min.	Max.	Mean
P—1	2.36	20 hr.	3.9	5.6	4.9
P—4	3.06	8 hr.	3.7	7.2	5.1
P—5	4.25	7 hr.	2.4	3.0	2.7
P—7	2.30	2 d.	1.8	4.3	3.5
P—9	3.36	13 hr.	2.9	7.3	5.1
P—12	3.30	23 hr.	1.2	7.3	3.7
P—14	3.08	2 d.	2.0	6.0	3.9
P—17	1.86	33 hr.	2.6	8.1	4.7
P—20	2.92	3 hr.	1.7	5.8	3.6
P—26	2.82	34 hr.	3.2	10.7	5.9
P—27	2.99	34 hr.	3.5	10.2	6.6
P—30	3.66	6 d.	10.9	14.9	12.6
P—32	2.78	9 hr.	2.9	7.3	4.4
P—33	3.06	11 hr.	4.8	7.5	6.0
P—34	2.38	15 hr.	5.3	6.9	6.1
P—35	3.77	8 hr.	3.8	6.1	5.0
P—38	2.44	9 hr.	4.4	8.1	5.4
P—41	3.01	2 d.	3.9	7.7	5.4
P—42	3.47	3 d.	1.6	7.0	4.5
P—44	2.70	12 hr.	5.9	9.8	7.1
P—45	2.91	16 hr.	4.8	6.2	5.3
P—46	3.38	6 hr.	2.8	6.2	3.8
P—47	2.53	4 hr.	2.3	4.6	3.3
P—48	2.75	4 hr.	4.5	6.9	5.6
		22 hr.	2.1	3.6	2.9
P—51	3.28	22 hr.	2.9	7.9	5.3
P—53	3.46	8 hr.	2.9	6.5	4.0
		2 d.	3.8	6.7	5.2
		6 d.	3.1	6.2	4.6
P—54	3.08	21 hr.	2.8	5.8	3.9
		2 d.	2.9	8.1	5.2
P—55	3.45	3 d.	3.9	6.5	5.3

hours from five infants supported the assumption that measurements made immediately after a gas collection were apparently applicable to the collection period itself. Alveolar P_{CO_2} (P_{ACO_2}) was considered to be in equilibrium with the "arterialized" capillary P_{CO_2}. CO_2 production ($\dot{V}_{CO_2}$) and O_2 consumption ($\dot{V}_{O_2}$) were calculated from the gas analyses and collection volumes using the standard respiratory equations (5). Alveolar ventilation ($\dot{V}_A$) and functional dead space (V_D) were secondarily derived from the following equations:

$$\dot{V}_A = \dot{V}_{CO_2} \div \frac{P_{ACO_2}}{P_B - 47}$$

where P_{ACO_2} is assumed to be equal to P_{aCO_2};

$$V_D = \frac{\dot{V} - \dot{V}_A}{f}$$

The equation for V_D is merely an adaptation of the Bohr equation for dead space.

P_{ACO_2} = partial pressure of CO_2 in alveolar gas in mm. Hg

P_{aCO_2} = partial pressure of CO_2 in arterial blood in mm. Hg

P_B = atmospheric pressure in mm. Hg

47 = partial pressure of water vapor in alveolar gas in mm. Hg

Since alveolar ventilation is related to the rate of excretion of CO_2 by the lungs, it has a physiologic connotation. Consequently functional dead space, derived by subtracting tidal alveolar ventilation from tidal volume, is also a physiologic concept. In adults the space thus calculated corresponds quite closely to the "anatomic" dead space (17).

The work of respiration was estimated from a simplified formula proposed by McIlroy:

$$\text{Work} = 0.6\,P\,\dot{V}, \text{ in Gm. cm.}$$

where P = the difference in cm. of H_2O between maximal and minimal pressures occurring during respiration.

This formula is based on the fact that the work of a purely elastic system would be represented by the equation $0.5\,P\,\dot{V}$ (the area of a triangle) and the work of a purely viscous system, by the equation $0.79\,P\,\dot{V}$ (the area of one half an ellipse). In normal adults (14) and infants (18) between 60 and 70 per cent of the work of respiration is expended against elastic resistance. Thus the constant 0.6 appears to be a reasonable figure and actually provides a good approximation of the work of normal respiration in infants when compared to more detailed methods for calculation of work (18).

RESULTS

The results of the studies are presented in Tables I, II, and III, and Figures 2 and 3. The minute volumes,[8] rates and tidal volumes listed

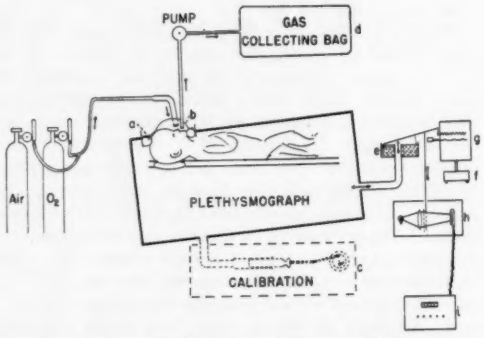

FIG. 1. DIAGRAM OF APPARATUS USED FOR RESPIRATORY STUDIES

a, pneumatic cuff; b, plastic face mask sealed to cuff; c, syringe and pump used for calibration of volume recorder; d, plastic bag for collecting gas containing expired air; e, Krogh spirometer with writing pen; f, kymograph; g, electric timer; h and i, photoelectric volume recorder.

[8] It is of interest that when a body weight of 70 Kg. is substituted in the regression equations for minute volume and alveolar ventilation calculated from the infant data (Figures 2 and 3), the resulting volumes approximate the actual adult values (Table IV).

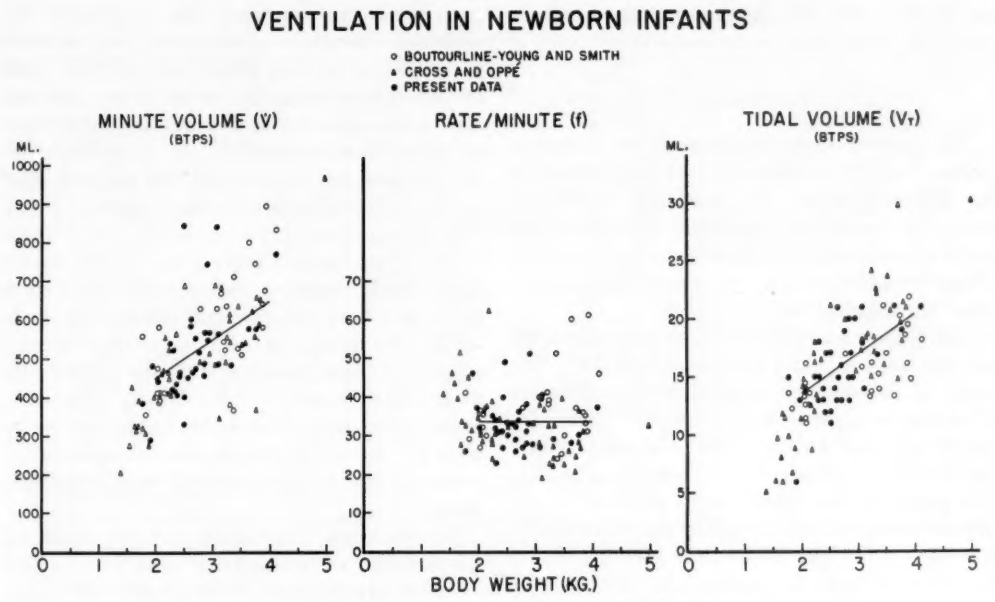

FIG. 2. MINUTE VOLUME, RATE AND TIDAL VOLUME OF 107 NORMAL INFANTS

The regression lines are plotted for the weight range of 2.00 to 4.00 Kg. from the equations: $\dot{V}$, in ml. = 251 + 99·body wt. in Kg.; f = 33.6 − 0.11·body wt. in Kg.; V_T, in ml. − 6.79 + 3.39·body wt. in Kg.

in Table I are compared on a weight basis in Figure 2 with those obtained for infants in the same age group (under seven days) by Cross and Oppé (2, 3) who used a similar technique, and with those reported by Boutourline-Young and Smith (1) who used a plethysmograph and a neck seal. The minute volumes from all three series appear comparable but the rates from Boutourline-Young and Smith's report are slightly higher and tidal volumes slightly lower, discrepancies which may be related to the different methods used. In any case a significant direct relationship between both minute volume and tidal volume on the one hand and body weight on the other is obvious. In the weight range studied there is no correlation between rate and body weight.

In the 16 infants in whom additional measurements allowed calculation of alveolar ventilation [8] and functional dead space (Table II and Figure 3), a direct relationship between these derived values and body weight is also suggested. The ratio of dead space to tidal volume ranged from 0.13 to 0.55 (average, 0.32), without relationship to weight or age. Oxygen consumption values for these 16 infants are also included in Table II.

Average intraesophageal pressure changes (ΔIEP) found during 32 studies of 28 normal infants (mean for group, 5 cm. water) show no apparent relationship to weight or age over a range of 1.86 to 4.25 Kg. birth weight (Table III) and

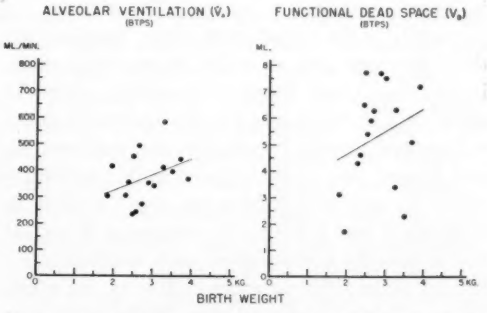

FIG. 3. ALVEOLAR VENTILATION AND FUNCTIONAL DEAD SPACE OF 16 NORMAL INFANTS

The regression lines are plotted from the equations: $\dot{V}_A$, in ml. = 208 + 59·body wt. in Kg.; V_D, in ml. = 2.84 + 0.88·body wt. in Kg.

are in fact, with one exception, in the range of 3 to 8 cm. water found in adults (19).

DISCUSSION

The present study confirms earlier measurements of minute volume, rate and tidal volume in the newborn infant. By providing further information previously unavailable on related aspects of respiration, it extends the categories in which comparisons may be made with normal adult physiological data.

The following comparisons have been calculated on the basis of basal metabolism (BMR)[9] because of uncertainties involved in the estimation of surface area, particularly in infants. Values for the 70 Kg. adult (BMR = 1610 Cal. per 24 hrs.) are shown in Column A of Table IV (19–22). For predicting the volume event values for a 2.5 Kg. infant in Column B an average heat production of 115 Cal. per 24 hrs. has been chosen on the basis of previous reports (23, 24) and the V_{CO_2} and V_{O_2} determinations of this study. Where respiratory rate is involved in the calculations, Column B is divided into two sets of predicted values, one employing the adult rate of 12, and the other the infant rate of 34. Column C shows the average values for a 2.5 Kg. infant based on actual results of the present investigation.

Comparison of Columns B and C shows that the predicted and actual values for minute volume are quite similar. The predicted alveolar ventilation is slightly lower than the determined value, a fact presumably related to the lower P_{CO_2} in infants compared to adults. The V_T and V_D predicted for the hypothetical infant breathing 12 times a minute are, as might be expected, much larger than those found. However, when the actual rate of 34 is applied to the predicted minute volume and alveolar ventilation, the resultant V_T and V_D are close to those found in this investigation. As can be seen from the V_D/V_T ratios in Columns A and C the rapid respiration of the infant is actually accomplished with ventilatory efficiency approximately equal to that of the adult. This small functional dead space in the infant may be secondary to (a) functioning respiratory epithelium in bronchi or bronchioles, (b) increased gas diffusion between alveoli and alveolar ducts or (c) relative reduction in the actual anatomic dead space. Concerning the first two possibilities, no information is available. In connection with the third, the few measurements of anatomic dead space (excluding nose and nasopharynx) in term infants range from 2.5 to 10 ml. (25, 26). Application of information from adults to the smaller figure would suggest a total anatomic dead space of 5.0 to 7.5 ml. for the term infant, a figure in fairly close agreement with the average physiologic dead space calculated from the present data for a slightly smaller 2.5 Kg. infant. Obviously more studies of total anatomic dead space in infants are needed for an adequate comparison between volumetric measurements and physiologic values.

An additional explanation for the rapid respiratory rate of the newborn infant may be provided by measurements of pulmonary compliance, elastic and viscous resistances and the work of respiration. Otis, Fenn, and Rahn (27) have calculated from such measurements the rate at which the "minimal work of respiration" would occur in the normal adult. Preliminary determinations and calculations in this laboratory thus far indicate that this theoretical minimum in infants may occur at the rates usually encountered. Certainly the newborn infant can increase his depth of breathing, as in many of those studied much larger tidal volumes were frequently observed in sighs or during restlessness before or after the basal periods selected for analysis. Nevertheless, the more rapid and shallow respiration may be a mechanically advantageous adaptation to physical factors peculiar to the lungs and chest of the newborn infant. These factors are at present under investigation in both the normal and abnormal infant.

The functional residual capacity (V_{FRC}) of a 2.5 Kg. infant calculated from measurements recently made by one of us (P. K.) (28) has been included in Table IV. The discrepancy between this figure of 70 ml. and the 193 ml. predicted from the data obtained in adult subjects requires comment. Compared on the basis of weight, the adult's lung has more than double the functional residual capacity of the infant's lung (3.7 com-

[9] Actually, if the 2.5 Kg. infant is assumed to have a surface area of 0.17 M^2., adult and infant data may be compared on the basis of surface area with results similar to those in Table IV.

TABLE IV

Comparison of adult and infant respiratory data

	Column A 70 Kg. Adult (19–22)	Column B 2.5 Kg. Infant (23, 24) (Predicted from adult data)		Column C† 2.5 Kg. Infant (Present data)
BMR (Cal./24 hrs.)	1,610	115		115
$\dot{V}_{O_2}$ (ml.)*	232	16.6		17
$\dot{V}_{CO_2}$ (ml.)*	200	14.3		12.3
$\dot{V}$ (ml.)	6,000	430		498
f	12	12	34	34
V_T (ml.)	500	36	13	15
$\dot{V}_A$ (ml.)	4,140	296		355
V_D (ml.)	155	11	4	5
V_D/V_T	0.31	—		0.32§
$\dot{V}_{O_2}/\dot{V}_A$	0.067	—		0.062§
ΔIEP (cm. H_2O)	4.7	—		5.0
Work/min. (Gm. cm.)‡	16,900	1,210		1,450
V_{FRC} (ml.)	2,700	193		70
$\frac{V_T - V_D}{V_{FRC}}$	0.13	0.13	0.05	0.13

* These volumes are STPD; all others, BTPS.

† $\dot{V}$, f, and V_T are derived from Figure 2; the V_{FRC} is calculated from P. K.'s data (28); the other volume values are calculated from the data of Table II; the ΔIEP value is derived from Table III.

‡ Work calculated from formula, 0.6 $P\dot{V}$ (18).

§ These ratios represent averages from Table II.

pared to 1.4 ml. per gram), a significant difference in expansion presumably related to the obvious differences in mechanical properties of the chest wall and lungs. Nevertheless, the similarity of the infant and adult $(V_T - V_D)/V_{FRC}$ ratios shown in Table IV suggests that the effective ventilation of each breath bears the same relation to functional residual capacity in the infant as in the adult. This is evidence of fundamental functional similarity between infant and adult lungs in spite of certain rate, volume and mechanical differences.

The present data have shown that ΔIEP and therefore, presumably, the intrapleural pressure change during respiration for the adult and for the infant is approximately the same. The actual work of respiration, as calculated from the minute volume and ΔIEP measurements corresponds closely to the predicted value in Column B. The possibility that this work may be normally performed at rates allowing minimal expenditure of energy by the infant has been considered above. In any case, if the mechanical efficiency of the respiratory muscles is estimated to be between 5 and 10 per cent, as in adults (27), the work of quiet respiration in the infant is apparently between 0.5 and 1.0 per cent of the total basal metabolism.

SUMMARY AND CONCLUSIONS

1. Measurements of respiratory minute volume, rate and tidal volume are presented for 35 normal newborn infants (birth weight, 1.82 to 4.08 Kg.) with determinations of the alveolar ventilation and functional dead space in 16. On the basis of these studies the expected averages for a normal infant of 2.5 Kg. during the first week of life are approximately: minute volume, 498 ml.; rate, 34; tidal volume, 15 ml.; alveolar ventilation, 355 ml.; functional dead space, 5 ml. The average values for infants of different sizes varied directly with body weight, with the exception that rate tended to be slightly more rapid in the smaller infants.

2. Intraesophageal pressure differences during respiration were determined in 28 infants as an index of intrapleural pressure changes. The average differences between maximal and minimal pressures for individual infants showed no relationship to weight or age and, with one exception, ranged from 3 to 8 cm. H_2O pressure (mean, 5 cm.). Work of respiration has been estimated to be approximately 1450 Gm. cm. per minute for the normal 2.5 Kg. infant.

3. Comparison, using basal metabolic rate as the common denominator, of the present data for infants with that available for normal adults indi-

cates essential similarity of minute volume, alveolar ventilation and work of respiration. The determined tidal volume and functional dead space of the infant are smaller than predicted from the adult values. However, when the actual more rapid respiratory rate of the infant is used in the calculations, the determined and predicted values are similar. The functional residual volume in the infant appears significantly lower than that of the adult when compared on the basis of metabolism.

4. The suggestion is advanced that the comparatively rapid respiration in the infant is not only possible because of a small functional dead space but probably optimal because of elastic and viscous properties of the lung peculiar to the newborn.

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PREPARATION OF A STABLE HUMAN PLASMA PROTEIN SOLUTION [1]

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Studies on the effect of heat on the virus of serum hepatitis have shown that a mixture of normal serum albumin and proved icterogenic plasma failed to produce serum hepatitis when inoculated into volunteers after it had been heated at 60°C. for 10 hours (1). Studies by Murray and Diefenbach (2) on the effect of heating infected plasma pools showed that after four hours heating at 60°C. the infected plasma pools retained their ability to produce hepatitis. A statement was made in their paper that heating for four hours or more at 60°C. led to an increase in turbidity and formation of a precipitate (2). With these two facts in mind plus the fact that a plasma protein solution heated for 10 hours at 60°C. might be clinically useful, we decided to investigate the possibility of preparing a plasma protein solution that could stand heating for 10 hours at 60°C. with little or no change in the proteins.

The present communication concerns the heat stability of the human plasma protein solution remaining after the crude fibrinogen and gamma globulin fractions have been separated from human plasma by ethanol-water mixtures at low temperatures.

METHODS AND RESULTS

The procedure for the separation of the crude fibrinogen fraction, Fraction I and the crude gamma globulin fraction, Fraction II + III was in part similar to that using ethanol at low temperatures (3). Fractions I and II + III were removed from the starting plasma for three primary reasons. First, the proteins contained in these two fractions have only a minor role in the colloid osmotic pressure of plasma. Second, the proteins fibrinogen and gamma globulin, contained in Fractions I and II + III, respectively, could be salvaged for their specific clinical uses; *i.e.*, afibrinogenemia, hemophilia and other coagulation component defects, agammaglobulinemia and specific infections requiring prophylaxis with antibodies. Third, Fractions I and II + III contain most of the more heat labile components of human plasma. The supernatant fluid following the removal of Fractions I and II + III was dried from the frozen state. The powder obtained was the material used in this investigation.

Plasma used in the preparation of this material was supplied by the American Red Cross through its Regional Blood Centers at Omaha, Nebraska, and Springfield, Missouri. The plasma was pooled at the Blood Center into two-liter bottles and ten liters were shipped to us each week. No attempt was made to keep the plasma cold while en route. Upon arrival at the laboratory each bottle was placed in a freezer kept at −18°C. When ready for processing, the plasma was thawed in a bath maintained at 37°C. and the separation of Fraction I and II + III was started immediately.

Separation of Fraction I

Fraction I was separated by 8 per cent ethanol, pH 7.2 ± 0.2, $\Gamma/2 = 0.14$, and temperature 0°C. ± 0.5°C.

To each liter of plasma, 92 ml. of cold 95 per cent ethanol were added slowly by siphon at a rate of 50 ml. per minute. The temperature was maintained at 0°C. ± 0.5°C. Adjustment of the pH to 7.2 ± 0.2 with 0.1 M bicarbonate was necessary as the pH of the starting plasma was usually around 6.8. The precipitated Fraction I was removed by centrifugation in a small Sharples centrifuge at 0°C. ± 0.5°C., at a feed rate of 50 ml. per minute.

Separation of Fraction II + III

Fraction II + III was separated by 25 per cent ethanol, pH 6.8 ± 0.05, $\Gamma/2 = 0.12$ and temperature −5°C.

To each liter of the supernatant fluid remaining following removal of Fraction I, 234 ml. of cold 95 per cent ethanol containing a predetermined amount of pH 4 acetate buffer [2] was added by siphon at a rate of 50 ml. per minute. The temperature was lowered to −5°C. during the addition of the reagent. The precipitated Fraction II + III was removed in the Sharples centrifuge at −5°C. at a feed rate of 30 ml. per minute.

The supernatant fluid following removal of Fraction II + III was clarified by filtration at −5°C. through

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[2] This buffer has a mole ratio of sodium acetate-acetic acid of 0.2 and is conveniently made up by taking: 200 ml. of 4 molar sodium acetate; 400 ml. of 10 molar acetic acid and water to make 1 liter. When diluted with water eighty times, it should have a pH of 4.00 ± 0.02 when measured in a glass electrode at 25°C.

TABLE I

The electrophoretic distribution of the proteins of the supernatant fluid following drying and re-constitution in pH 8.6 veronal citrate buffer, Γ/2 = 0.1

Prep. No.	Yield (approx. dry wt. per liter of plasma) *grams*	Protein content of dry weight *per cent*	Electrophoretic distribution				
			Albumin *per cent*	α-globulin *per cent*	β-globulin *per cent*	Fibrinogen *per cent*	γ-globulin *per cent*
1	59	60.4	80.3	14.1	5.6	0	0
2	56	65.2	82.7	11.7	5.6	0	0
3	57	62.8	81.7	12.6	5.7	0	0

Hormann D-8 filter pads with the aid of Hyflo Super Cel.[3] The fluid so clarified was then dried from the frozen state and stored at − 18°C. until used.

Electrophoretic distribution of the powder obtained from drying the supernatant fluid

The protein distribution of three different preparations made by the procedure above has been determined using the Klett Electrophoresis apparatus. The material for the electrophoresis determination was dissolved to a concentration of 1 to 2 per cent with veronal citrate buffer of 0.1 ionic strength and pH 8.6. Table I shows the protein distribution for each preparation. Preparation 1 represents a mixture of the powder obtained from six, four-liter plasma pools. Preparations 2 and 3 represent the powders obtained from 8.8 and 8.1-liter plasma pools, respectively.

The table shows that the yield of the dry powder from the supernatant fluid in each preparation was between 56 to 59 grams per liter of plasma. This weight was not adjusted for moisture content which is usually between 5 to 10 per cent in our method of drying from the frozen state. The protein content of the powder based on the biuret determination (4) was between 60 and 65 per cent. The protein distribution of the powder was 80 to 83 per cent albumin, 11 to 14 per cent alpha globulin, and 5.6 to 5.7 per cent beta globulin. Neither fibrinogen nor gamma globulin was contained in this material.

The effect of heating for 10 hours at 60°C. on the optical density of the plasma protein solution

The powder obtained from drying the supernatant fluid following the removal of Fractions I and II + III was reconstituted to a five per cent solution with cold distilled water. The pH of the solution was adjusted to 6.8 ± 0.05 when necessary. The protein concentration was 3.3 per cent as determined by the biuret method (4).

The reconstituted material was sterilized by filtration through Hormann D-3, D-8 and D-10 filter pads, placed into sterile Coleman, Jr. Spectrophotometer tubes, and capped with sterile rubber stoppers. Some of the tubes were heated for 10 hours at 60°C., while others not heated were used as control tubes. Optical density readings of both the heated and control tubes were made at 550 mμ in the Coleman Jr. Spectrophotometer. Table II shows that heating for 10 hours at 60°C. changed the optical density very little.

The ultracentrifugal and electrophoretic analyses of the unheated solution and of the solution heated for 10 hours at 60° C.

Heated and unheated samples of Preparations 1, 2, and 3 were studied in the Spinco Ultracentrifuge Model E. One ml. of each preparation was diluted with 2 ml. of 0.15 M NaCl making an average protein concentration of 1.1 per cent. For each preparation the heated and unheated samples were run at the same time using both cells of the rotor. Each sample was run at full

TABLE II

The effect of heating for 10 hours at 60°C. at pH 6.8 on the optical density of the plasma protein solution

Preparation number	Heat treatment	Number tubes read	Average optical density
1	Control	25	0.203
	10 hours at 60°C.	25	0.209
2	Control	20	0.174
	10 hours at 60°C.	50	0.190
3	Control	20	0.192
	10 hours at 60°C.	49	0.200

[3] Hyflo Super Cel was suspended in the supernatant fluid to approximately 0.25 per cent. The filter pads were precoated with Super Cel also.

speed of 59,700 RPM for 100 minutes and pictures were taken at 5, 10, 15, 20, 30, 40, and 100 minutes after the rotor had reached full speed. Five minutes after the rotor had reached full speed a fast moving component was observed in the heated sample of the three preparations (arrow, upper diagram, Figure 1A). Very little of this component was seen in the unheated sample (lower diagram, Figure 1A). Five minutes later this component was hardly discernible (arrow, upper diagram, Figure 1B). Throughout the remainder of the run no components other than the main component in both the unheated and heated samples were seen in the ultracentrifuge pattern.

To ascertain to what extent the main component was decreased due to heating for 10 hours at 60°C., the area of the main peak of each of the heated and unheated samples was determined from the picture taken 100 minutes after full speed had been attained (Figure 1C). The areas of the main peaks of the heated samples compared with those of the unheated samples in Preparations 1, 2, and 3 were approximately 86.2, 96.0, and 83.4 per cent, respectively. This indicates that heating causes a reduction in the amount of the main component. Whether the reduction in the main component is accounted for in the fast component has not been determined. The uncorrected sedimentation constant of the main peaks in each of the heated and unheated samples was nearly the same in all preparations, approximately 4×10^{-13}.

The electrophoretic results obtained on the heated solution of Preparations 1, 2, and 3 showed the protein distribution to be very nearly the same as that in the starting material (Table I). Albumin was approximately 80 per cent, alpha globulin 16 per cent, and beta globulin 4 per cent.

DISCUSSION

By the use of the cold ethanol method (3, 5), Pennell (6) has fractionated plasma to which either T6 phage virus or mouse SK polio virus had been added. He showed that for the most part both viruses were removed in Fractions I and II + III. The supernatant fluid following removal of Fraction II + III contained only a small percentage of the starting concentration of either virus. Murray (7) reported that albumin prepared by the cold ethanol method from a human plasma pool infected with homologous serum hepatitis was not infective in 10 volunteers when it had been heated for 10 hours at 60°C. When 100 ml. of 25 per cent albumin not heated for 10 hours at 60°C. were given to each of five volunteers, one case of hepatitis developed. Murray and Ratner (8) have demonstrated further that gamma globulin prepared by the cold ethanol method from human plasma infected with homologous serum hepatitis does not produce hepatitis in humans.

The work of Pennell and Murray suggests that both the cold ethanol fractionation process and the heating for 10 hours at 60°C. are responsible for the albumin solution being free of virus. The

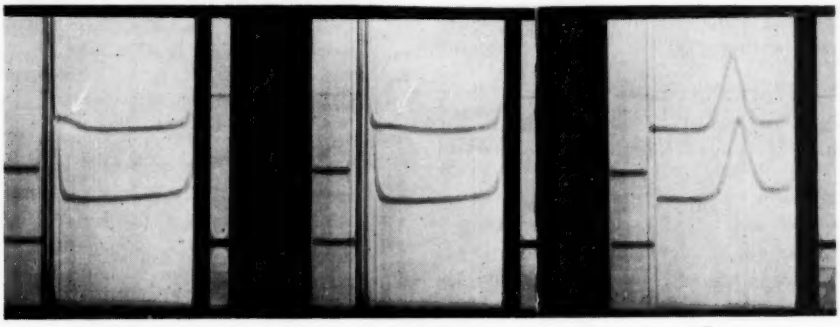

FIG. 1. THE ULTRACENTRIFUGAL SEDIMENTATION DIAGRAMS OF THE PLASMA PROTEIN SOLUTION, PREPARATION 3

The upper diagrams represent the heated solution, the lower the unheated solution. Picture A was taken 5 minutes after reaching full speed (59,700 RPM); B, 10 minutes after full speed; and C, 100 minutes after full speed.

method described in this communication makes use of the cold ethanol process to remove the more unstable proteins thus permitting heating for 10 hours at 60°C. A study of this process on plasma infected with homologous serum hepatitis is indicated. If proven successful in human volunteers, the plasma protein solution resulting could be prepared on a large scale more economically than can albumin with equipment that already exists in the industrial laboratories.

Preliminary studies suggest that acetyl-dl-tryptophanate is a stabilizing agent for the plasma protein solution. The present method is being studied on a pilot plant scale by the Biologic Laboratories, Massachusetts Department of Public Health, Boston. Large volumes of the solution containing acetyl-dl-tryptophanate are being heated for 10 hours at 60°C.

SUMMARY

1. A method for the preparation of a stable human plasma protein solution involving the removal of the more heat labile components from plasma with ethanol-water mixtures has been described.

2. The solution contains nearly all the albumin and some of the small molecular weight globulins that are normally contained in plasma.

3. Very little, if any change is observed in the optical density and electrophoretic distribution of the solution following heating for 10 hours at 60°C.

4. Ultracentrifugal analyses show the formation of a fast moving component in the solution due to the 10 hours' heating at 60°C.

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THE PHYSIOLOGIC CLOSURE OF THE DUCTUS ARTERIOSUS IN THE NEWBORN INFANT [1, 2]

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The exact time of functional closure of the ductus arteriosus in the newborn human infant has remained a matter of speculation for many years. Studies of human anatomical specimens have shown that complete morphologic closure requires from four to eight weeks (1–5).

Animal experiments have been the main approach to the study of functional closure. All workers agree that at the time of birth, but before the onset of respiration and inflation of the lungs, the pressure in the pulmonary artery is equal to or greater than the pressure in the aorta and that a considerable portion of the output of the right ventricle passes through the ductus into the descending aorta. Most workers agree that in animals, with the onset of respiration, there occurs, within a few minutes, a rapid fall in pulmonary artery pressure so that blood no longer flows through the ductus into the aorta. However, Everett and Johnson (6) by injecting radioactive material into the right ventricle and studying the amount appearing in the dorsal aorta distal to the ductus in newborn puppies felt that the flow through the ductus into the aorta may persist for as long as nine hours after birth.

There is less agreement about the mechanism of this early functional closure of the ductus. Kennedy and Clark (7–9) observed prompt closure of the guinea pig ductus by muscular contraction during oxygen breathing, but Barron (10) could not corroborate this effect of oxygen in the lamb fetus. Other workers who have studied the closure of the ductus in the newborn lamb by angiography suggested that prompt closure occurred by muscular contraction within a few minutes after birth and that occasionally, after initial closure, the ductus may reopen (11–13).

Recently careful studies in lambs have been made by Dawes, Mott, Widdicombe, and Wyatt (14) and Ardran, Dawes, Prichard, Reynolds, and Wyatt (15). They employed the technic of cineangiography as well as intravascular pressure measurements and blood oxygen analyses. An immediate fall in pulmonary artery pressure and pulmonary vascular resistance accompanied by a great increase in the velocity of blood flow through the pulmonary circuit was found to occur with inflation of the lungs. The fall in pulmonary artery pressure was accompanied by a reversal of the flow through the ductus. The ductus was not observed to close during the course of these observations which lasted up to 90 minutes after birth.

Fewer experimental data are available regarding the closure of the ductus in the human. This, however, has not impeded the free application of results of animal experiments to the human despite words of caution by careful investigators (12). However, it is generally agreed that, as in animals, a physiologic pulmonary hypertension is present in the human fetus at the time of birth and before inflation of the lungs, but little is known about the changes in the circulation through the ductus and the lungs that occur with the onset of respiration. Patten (4, 16) has suggested that the circulatory adjustments in the human at birth may be more gradual than the abrupt alterations that occur in animals. This concept is based upon careful post-mortem anatomic measurements made in a large number of stillborn fetuses and newborn infants which demonstrate a well-developed pulmonary circulation at the time of birth and a gradual transition in the size of the vessels to adult

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[3] Evelyn Neizer Fellow in Medicine.

[4] Scholar in Medical Science—John and Mary R. Markle Foundation.

relationships during the first two to three months of life.

The most important experimental studies of the closure of the human ductus have utilized the technic of angiography. Using retrograde aortography, Keith and Forsyth (17) found in two infants five days of age that no contrast medium entered the ductus from the aorta and concluded that functional closure was complete. Using venous angiography, Lind and Wegelius (18) found the ductus to be patent in six infants immediately after birth, and in "some other instances to have closed a few hours after birth." Retrograde aortography in two infants, two and six hours after birth, disclosed no contrast medium going through the ductus from the aorta into the pulmonary artery. However, in a later presentation (19) Lind described the results of venous angiography in 10 newborns studied within 12 hours after birth. In none of these cases was the ductus arteriosus visualized. Johnsson (20) noted one instance of a cyanotic newborn infant in whom ductal flow from pulmonary artery to aorta was demonstrated by angiography, and in whom cyanosis had disappeared one week later at which time angiography revealed no veno-arterial ductal shunt.

These conflicting results indicate that the exact time of functional closure of the human ductus is still unsettled but that, at least in some infants, the ductus may be patent during the first few hours of life and that it may be the site of a veno-arterial shunt of considerable magnitude. The present study was stimulated by these studies and by the observation that infants in the neonatal period occasionally show cyanosis of the lower part of the body but not of the upper portion. Regional cyanosis of this type has been observed in older children and adults who have a patent ductus arteriosus with pulmonary hypertension and a veno-arterial or "reversed" shunt through the ductus (21, 22). Due to this shunt of venous blood through the ductus to the descending aorta the arterial oxygen saturation is lower in the feet than in the hands. We believed that if a similar shunt were present in the newborn, it would continue as long as the ductus remained patent and the pressure in the pulmonary artery exceeded that in the aorta. To examine this possibility we have carried out 66 studies on 60 infants of varying ages. The method of study was based upon determinations of the oxygen content of blood samples obtained from the upper and lower extremities.

METHODS

Arterialized capillary blood samples were collected from a finger of the right hand and from the toe of either foot. The right hand was selected rather than the left since occasionally the orifice of the left subclavian artery may lie opposite the ductus and, in the presence of a ductal veno-arterial shunt, allow some unsaturated blood to enter the left arm (23). The technic of sampling has been previously described (24). The extremities were warmed by hot moist compresses for five minutes and a deep incision made in the fleshy tip of the digit by a sharp scalpel blade. This usually produced from 0.5 to 1.0 ml. of blood in about 60 seconds. The samples were then chilled and analyses made within a few hours for oxygen contents and capacities by the Roughton-Scholander micro gasometric method for estimation of blood oxygen (25). Oxygen capacities were determined in all but 5 samples, where the quantity was insufficient to perform the analysis. In these specimens the oxygen capacity was calculated from the hemoglobin content of the sample. Estimated values for dissolved oxygen were subtracted from these figures, and the results expressed in terms of per cent oxygen saturation. Samples collected and analyzed in this manner have been shown to be identical in oxygen content to simultaneously drawn arterial samples (24). The blood thus obtained probably comes from small arterioles or arteries since the blood flow is rapid and occasionally can be seen to "spurt" in synchrony with the radial pulse, and if a blood pressure cuff placed around the arm is inflated to diastolic pressure during the warming of the hand and the sampling period, the hematocrit and oxygen saturation of the sample are unchanged (26). This would not be the case if capillary or venous blood were being obtained.

Most infants cried during the sampling period and were indeed encouraged to cry because continuous crying would minimize the gross differences in ventilation which might occur in the change from quiet to crying state. Since the samples were not taken simultaneously and in order to randomize the effect of crying on the samples, the foot and the hand were alternated as the site of the first sample. Analysis of the data obtained revealed no variations in hand-foot oxygen saturations that could be ascribed to whether the hand or foot sample was obtained first.

As part of the study, a careful auscultation of the heart was performed on all infants, and a note made of the presence or absence of cyanosis of any part of the body.

OBSERVATIONS

In 57 out of the 66 studies the blood sample from the right hand had an oxygen saturation of

90 per cent or more (Tables I and II). This indicates that true arterial blood had been obtained and that the sampling technic and methods of analysis were adequate. The nine cases in which an oxygen saturation of less than 90 per cent was obtained from the right hand showed levels only slightly below 90 per cent. They were: 87.7, 88.8, 87.0, 86.0, 87.4, 89.8, 86.6, 88.8, and 85.4 per cent saturation. The ages of these infants were 40 minutes, 1½ hours, 17½ hours, 20½ hours, 6½ months, 8 months, 9 months, 10 months, and 10 months, respectively.

A comparison of the blood oxygen saturation of the right hand with that of the foot in 62 studies

TABLE I

Hand-foot oxygen saturation differences and magnitude of ductal veno-arterial shunt in 43 studies performed on 37 infants within five days after birth *

Infant	Age *hours*	Right hand: Oxygen content *ml./100 ml. blood*	Right hand: Oxygen capacity *ml./100 ml. blood*	Right hand: Oxygen saturation *per cent*	Foot: Oxygen content *ml./100 ml. blood*	Foot: Oxygen capacity *ml./100 ml. blood*	Foot: Oxygen saturation *per cent*	Difference in % saturation Hand minus foot +Foot saturation lower −Foot saturation higher	Significant veno-arterial shunt % of descending aorta flow (estimated)
1	⅔	19.35	22.13	87.7	19.73	22.47	87.4	+ 0.3	—
2	1	21.51	22.77	94.4	21.06	23.33	90.4	+ 4.0	—
3	1	20.04	22.22	90.3	19.59	23.43	83.5	+ 6.8	37
4	1½	23.11	25.13	92.0	22.22	25.48	87.2	+ 4.8	—
5	1½	18.86	21.22	88.8	19.76	24.15	81.8	+ 7.0	38
6	1½	23.98	25.68	93.4	22.19	26.29	84.4	+ 9.0	58
7	1½	22.09	23.42	94.4	21.36	23.86	89.4	+ 5.0	29
8	1⅔	17.42	18.90	92.2	16.15	19.36	83.4	+ 8.8	42
9	2	20.33	21.68	93.7	17.96	21.76	82.7	+11.0	59
10	2½	21.77	24.10	90.3	21.91	24.20	90.5	− 0.2	—
11	2½	24.63	26.96	91.4	23.52	26.96	87.3	+ 4.1	—
12	2¾	26.30	27.56	95.5	27.00	27.62	97.7	− 2.2	—
13	2¾	23.50	25.91	90.7	22.70	25.65	88.6	+ 2.1	—
14	3	23.68	24.62	96.2	23.03	24.12	95.6	+ 0.6	—
15	3½	27.68	29.08	95.2	25.63	28.08	91.3	+ 3.9	—
16	4½	24.40	26.12	93.5	23.99	26.19	91.6	+ 1.9	—
17	6	24.64	26.71	92.3	23.45	26.45	88.7	+ 3.6	—
18	6½	17.98	19.32	93.1	17.94	19.04	94.2	− 0.9	—
19	11½	22.19	24.31	91.3	22.34	24.38	91.5	− 0.2	—
20	12	18.02	19.28	93.5	17.93	19.69	90.8	+ 2.7	—
21	14½	26.65	28.88	92.3	27.62	29.45	93.8	− 1.5	—
22	17½	17.12	19.67	87.0	17.08	19.35	88.3	− 1.3	—
23	20	26.34	27.10	97.2	25.31	27.87	90.7	+ 6.5	43
24	21	19.18	22.29	86.0	18.76	22.29	84.0	+ 2.0	—
25	25	24.53	26.22	93.5	23.72	26.22	90.4	+ 3.1	—
26	27	22.55	24.92	90.5	20.72	25.12	82.5	+ 8.0	70
27	33	17.39	18.39	94.5	17.25	18.98	90.9	+ 3.6	—
28	42	22.80	24.15	94.3	23.05	25.12	92.0	+ 2.3	—
29 (9)	45	18.37	19.56	94.0	16.33	20.58	79.5	+14.5	72
30 (3)	47	20.70	22.43	92.2	18.71	22.91	81.7	+10.5	58
31 (6)	49	26.28	28.16	93.5	25.47	27.94	91.2	+ 2.3	—
32	50	23.78	25.35	93.8	23.61	25.20	93.7	+ 0.1	—
33	57	18.48	20.18	91.7	18.76	19.79	94.8	− 3.1	—
34	60	24.61	26.55	92.7	24.92	26.07	95.5	− 2.8	—
35	68	21.35	22.81	93.5	21.25	23.80	89.4	+ 4.1	—
36	81	21.46	23.85	90.0	21.88	25.03	87.3	+ 2.7	—
37	85	18.09	18.90	95.7	18.96	19.89	95.3	+ 0.2	—
38	94	26.49	28.53	93.0	28.27	29.97	94.3	− 1.3	—
39	96	25.39	27.14	93.6	24.53	26.05	94.1	− 0.5	—
40 (6)	103	22.87	24.59	93.2	22.24	24.03	92.5	+ 0.7	—
41	108	22.93	24.50	93.6	23.04	24.92	92.6	+ 1.0	—
42 (9)	117	18.15	19.80	91.7	17.70	19.37	91.3	+ 0.4	—
43 (2)	120	22.26	23.93	93.0	22.17	24.22	91.5	+ 1.5	—
Mean		22.08	23.84	92.6	21.65	24.10	89.7	+ 2.9	
Range		17.12 to 27.68	18.39 to 29.08	87.0 to 97.2	16.15 to 27.62	18.98 to 28.08	79.5 to 97.7	− 3.1 to +14.5	
Standard deviation		±2.96	±2.98	±2.3	±3.06	±2.92	±4.3	± 3.8	

* Repeat studies were performed in four infants. These are indicated by the number of the infant in parentheses following the repeat study.

TABLE II

Hand-foot oxygen saturation differences determined in 19 infants from 3½ to 11 months old (control group)

Infant	Age *months*	Right hand: Oxygen content *ml./100 ml. blood*	Right hand: Oxygen capacity *ml./100 ml. blood*	Right hand: Oxygen saturation *per cent*	Foot: Oxygen content *ml./100 ml. blood*	Foot: Oxygen capacity *ml./100 ml. blood*	Foot: Oxygen saturation *per cent*	Diff. in per cent saturation Hand minus foot +Foot saturation lower −Foot saturation higher
1	3½	13.58	15.08	90.1	13.34	15.08	88.6	+1.5
2	4	15.07	15.95	94.5	15.27	15.91	95.8	−1.3
3	4½	12.53	13.60	92.1	12.34	13.61	90.7	+1.4
4	4½	13.06	14.42	90.5	13.67	15.13	90.3	+0.2
5	4½	13.89	14.67	94.7	14.29	15.43	92.7	+2.0
6	5	14.55	15.60	93.3	14.28	15.11	94.6	−1.3
7	5	13.28	13.92	95.4	13.35	14.06	95.0	+0.4
8	5	13.76	14.40	95.5	12.97	14.17	91.6	+3.9
9	6	14.40	15.29	94.2	14.18	14.92	94.9	−0.7
10	6	10.20	10.74	94.9	10.57	11.19	94.3	+0.6
11	6½	11.14	12.76	87.4	10.88	11.84	92.0	−4.6
12	8	15.65	17.41	89.8	15.60	17.46	89.3	+0.6
13	8	14.40	15.97	90.2	14.35	16.31	88.0	+2.2
14	9	10.30	11.24	91.6	10.57	11.61	91.0	+0.6
15	9	13.51	15.60	86.6	14.71	16.66	88.3	−1.7
16	10	13.70	15.44	88.8	14.31	15.26	93.9	−5.1
17	10	12.68	14.86	85.4	13.82	15.93	86.7	−1.3
18	10½	16.33	17.47	93.5	16.22	16.96	95.7	−2.2
19	11	15.10	15.57	97.0	14.78	15.66	94.5	+2.5
Mean		13.53	14.74	91.9	13.66	14.85	92.0	−0.1
Range		10.20 to 16.33	10.74 to 17.47	85.4 to 97.0	10.57 to 16.22	11.19 to 17.46	86.7 to 95.8	−5.1 to +3.9
Standard deviation		±1.61	±1.70	±3.1	±1.58	±1.71	±2.8	±2.2

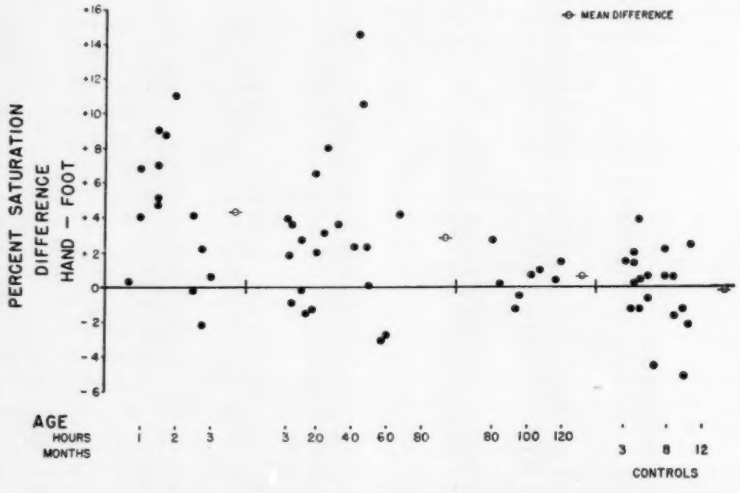

FIG. 1. GRAPHIC SUMMARY OF HAND-FOOT OXYGEN SATURATION DIFFERENCES IN 43 STUDIES ON 37 INFANTS PERFORMED WITHIN FIVE DAYS AFTER BIRTH COMPARED WITH CONTROL VALUES IN 19 OLDER CHILDREN

Positive values in this and subsequent figures indicate cases in which the oxygen saturation in the foot is lower than that in the hand. The mean difference for each group is indicated just to the right of the oldest infant in the group.

is shown in Tables I and II and graphically illustrated in Figure 1. For ease of presentation we have chosen to express the relationship between the oxygen saturations of blood from the upper and lower extremities as the hand saturation minus the foot saturation (hereafter referred to as the hand-foot difference); hence cases in which the foot saturation is lower will appear above the baseline of the graph and will have a positive value.

Nineteen infants aged 3½ months to 11 months who were free of cardiac or pulmonary disease that might affect arterial oxygen saturation served as controls (Table II). Since the ductus is anatomically closed by this age, all of these infants should have the same oxygen saturation in both hand and foot blood. In these cases, no hand-foot saturation difference of more than 5.1 per cent was observed.

A statistical evaluation of the data obtained from all four groups of infants studied is presented in Table III. It can be seen that there is a striking difference between the two groups of children less than 72 hours of age and the two groups of children older than 72 hours. The mean values are higher, and the variances are distinctly greater in the younger groups. The high variance (scatter) at the earlier ages is a reflection of the presence of a sizable fraction of the subjects giving large hand-foot differences, with many others giving near-zero values. The smaller variance in the later groups reflects the disappearance of high values, which may be interpreted as evidence of the disappearance of the ductal veno-arterial shunt within the first three days of life. The mean hand-foot difference for the 35 infants less than 72 hours old is 3.44; the mean hand-foot difference for the 27 infants older than 72 hours is .013. The difference between

TABLE III

Statistical evaluation of the data from the four groups of infants

Age group	Mean hand-foot difference % saturation	Standard error of mean	Variance of observations	Sample size No. infants
1 to 3 hrs.	4.37	1.04	15.244	14
3 to 72 hrs.	2.82	.94	18.73	21
72 to 120 hrs.	.59	.43	1.487	8
3½ to 11 mos.	−.10	.52	5.318	19

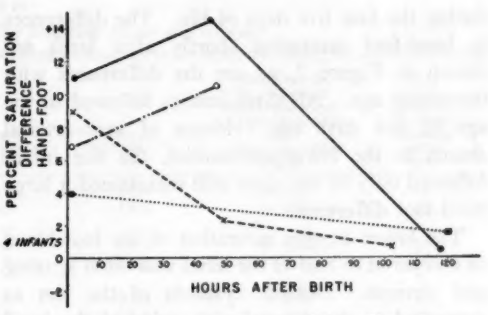

FIG. 2. SERIAL MEASUREMENTS OF HAND-FOOT OXYGEN SATURATION DIFFERENCES IN FOUR INFANTS

these two values is statistically significant at the .001 level of significance, and thus can hardly be attributed merely to chance factors. It is important to note that it is not only the mean which is changing but that also the variability amongst the hand-foot differences is much greater at earlier ages. The variances of the paired groups also differ far more than chance alone would explain, 1 per cent significance by Bartlett's test (27).

These data suggest the presence of a veno-arterial shunt through the ductus in a considerable number of infants during the first three days of life. If one accepts a 5 per cent hand-foot difference as probably certain evidence of a shunt (since only one of the 19 control cases had a difference larger than this), then it can be seen that 5 out of 14 infants or 36 per cent of cases under three hours of age and 4 out of 21 infants or 19 per cent of cases from 3 to 72 hours of age had veno-arterial shunts while none of the children in the 72- to 120-hour group exhibited evidence of such a shunt. It should be pointed out that a 5 per cent difference in saturation between hand and foot indicates the presence of a veno-arterial shunt contributing about 30 per cent of the total blood flow in the descending aorta. From inspection of the data in Figure 1 it is apparent that several infants probably had smaller shunts because the majority of the values in the first two groups of infants lie above the zero line of the graph while the values for the older children are more evenly scattered above and below the zero line. This is also reflected by the differences in the variances shown in Table III.

Four infants who initially presented evidence of a veno-arterial shunt were studied more than once

during the first five days of life. The differences in hand-foot saturation shortly after birth are shown in Figure 2, as are the differences with increasing age. All three infants followed to the age of five days lost evidence of veno-arterial shunts to the lower extremities, the one infant followed only to two days still maintained a large hand-foot difference.

The lower oxygen saturation of the foot blood as compared to that of the hand was often striking and obvious. Definite cyanosis of the feet as compared to the normal pink color of the hand was noted in almost all the infants with a hand-foot difference in oxygen saturation of 8 per cent or over. Upon obtaining the blood samples, inspection of the two syringes frequently revealed the obvious darker color of the less saturated sample from the foot.

Heart murmurs were carefully sought for in these infants. A murmur was heard in only one case, a one and one-half-hour-old girl who had a hand-foot oxygen saturation difference of 7.0 per cent. The murmur was systolic in time, of moderate intensity, and was heard best in the pulmonic area.

DISCUSSION

It may be remotely possible that the greater hand-foot oxygen saturation difference in the first few days of life may reflect regional differences in the circulation between these two extremities so that venous blood was obtained in the samples from the foot but not from the hand. However, no consistent differences in rate of flow were noted between the extremities; the differences in saturation had disappeared by the third day of life, and no differences in saturation were noted in the controls so that it would appear that the samples obtained were equivalent to samples of arterial blood. Since a patent ductus arteriosus with veno-arterial shunt is the most probable circulatory cause for the observed differences in hand-foot oxygen saturation, it is apparent that the above data have several important implications.

1. In the newborn human infant the ductus arteriosus is patent and is the site of a veno-arterial shunt of moderate to considerable magnitude for at least three hours after birth in about 30 per cent of infants, and in a smaller percentage of infants up to two or three days of age. Crude calculations, assuming a systemic arteriovenous oxygen difference of 4 ml. per 100 ml. blood, indicate that this veno-arterial shunt may contribute as much as 72 per cent of the total blood flow in the descending aorta The right hand column in Table I shows the estimated percentage of descending aorta flow contributed by the right-to-left shunt through the patent ductus in infants having hand-foot oxygen saturation differences of 5 per cent or more. In the six infants up to three hours of age who showed significant shunts, the range was from 29 to 59 per cent of the descending aorta flow with a mean for these infants of 44 per cent. In the four infants, 3 to 72 hours of age, who showed shunts, the range was from 43 to 72 per cent with a mean of 61 per cent. Because of the limits of error of the technic, veno-arterial shunts of less than 30 per cent of the total descending aorta flow could not be detected, and it may well be that a larger proportion of infants had shunts which were not detected in this study.

2. If a veno-arterial shunt occurs through the ductus, it follows that the pulmonary artery pressure must exceed or equal the pressure in the aorta as long as the shunt persists. Several studies (28, 29) have indicated that the femoral or brachial arterial pressure in the newborn infant is about 80/47 mm. Hg. If one subtracts 5 mm. from the systolic level to allow for the "standing pressure wave" in the femoral artery (30), the pressure in the aorta of the newborn can be approximated at 75/47 mm. Hg. Thus, the pulmonary artery pressure in the infants with ductal veno-arterial shunts must at some time in the heart cycle exceed this pressure although the magnitude of this difference in pressure is unknown. The size of the shunt and the pressure difference will also be related to the size of the ductus. With a relatively large ductus a large shunt may exist with little pressure difference. The influence of crying upon the pressure relationships and magnitude of the shunts is unknown. It is quite possible that the increase in intra-thoracic pressure associated with crying might cause a veno-arterial shunt to occur through the ductus where none was apparent at rest.

The cause of the disappearance of the veno-arterial shunt, which is complete in most infants by the age of 72 hours, is not clear. Two possibilities exist: (a) the ductus may have closed so

TABLE IV

Data on four infants before and after breathing 12 per cent oxygen

Infant	Age *hours*	Oxygen mixture	Oxygen saturation % Right hand	Oxygen saturation % Foot	Difference in % saturation Hand minus foot +Foot saturation lower −Foot saturation higher	Veno-arterial shunt % of descending aorta flow
A	4½	Room air	93.5	91.6	+ 1.9	—
		12% O_2	85.2	79.0	+ 6.2	39
B	6	Room air	92.3	88.7	+ 3.6	24
		12% O_2	79.7	73.1	+ 6.6	45
C	6½	Room air	93.1	94.0	− 0.9	—
		12% O_2	83.7	78.7	+ 5.0	24
D	14½	Room air	92.3	93.8	− 1.5	—
		12% O_2	57.4	47.3	+10.1	73

that it will not transmit blood in either direction, or (b) the pressure in the pulmonary artery may have fallen to a level below the aortic pressure thereby causing the veno-arterial shunt to disappear but making an arteriovenous shunt possible. The latter has been the case in the animal studies of Ardran and co-workers (15), where the pulmonary resistance and pressure fell markedly within 15 minutes of birth, thereby causing an arteriovenous shunt to develop.

To investigate these possibilities, hand and foot blood samples were collected in the manner described above from four infants before and at the end of a six-minute period of hypoxia. This was produced by having the infant breathe, by means of a face mask, a 12 per cent oxygen mixture. While breathing room air, there was no hand-foot difference of greater than 3.6 per cent in the infants studied (Table IV).

A significant degree of arterial unsaturation was produced by the low oxygen breathing since the oxygen saturation of hand blood fell in each case, the mean fall being 10 per cent, corresponding to a drop in oxygen tension of about 20 mm. Hg. In each case this resulted in an increase of the hand-foot difference indicating the re-establishment of the veno-arterial shunt through the ductus (Figure 3). One infant (Infant D, Table IV) had a marked generalized fall of oxygen saturation during the low oxygen breathing—from an initial value of 92 per cent saturation in the right hand to a value of 57 per cent. This was accompanied by visible, generalized cyanosis and somnolence. After room air breathing was resumed, the cyanosis promptly disappeared and no subsequent ill effects were noted. A similar precipitous fall in oxygen saturation in one patient was noted by Burchell, Swan, and Wood during the breathing of low oxygen mixtures by an older patient with a patent ductus and pulmonary hypertension (23). The mechanism of the response is unknown. Hypoventilation (31) or intense pulmonary vasoconstriction with a large veno-arterial ductal shunt associated with a right- to left-shunt through a patent foramen ovale are possibilities. Because of this hazard of induced hypoxia in infants, further studies were not carried out.

The above data suggest that the cause of the disappearance of the hand-foot oxygen saturation difference is a fall in pulmonary artery pressure

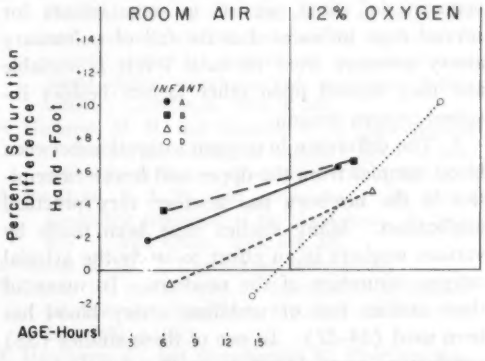

FIG. 3. EFFECT OF BREATHING 12 PER CENT OXYGEN UPON HAND-FOOT OXYGEN DIFFERENCES IN FOUR NEWBORN INFANTS

to a level below the aortic pressure rather than actual closure of the ductus arteriosus. The infant pulmonary vascular bed is apparently similar to that of the adult in its response to hypoxia, which causes an increase in pulmonary vascular resistance and a rise in pulmonary artery pressure. This is sufficient to re-establish the veno-arterial shunt through the ductus. Similar findings were noted by Lind and Wegelius (18) in two cases of neonatal asphyxia who gave angiographic evidence of a patent ductus with pulmonary artery to aorta flow. It is remotely possible that ductal closure has occurred by muscular contraction and that hypoxia has caused the ductus to reopen by muscular relaxation. One must still, however, assume that the pulmonary artery pressure has either remained higher than the aortic pressure since birth or that it has become elevated by the hypoxia. This problem will not be settled until further proof of the closure of the human ductus by muscular contraction is available. Whether muscular activity is involved in the process or not, these data raise the interesting question of the relationship of hypoxia to permanent patency, since there have been several reports of increased incidence of patent ductus arteriosus in children and adults at high altitudes where a consistently low oxygen tension is experienced (32, 33).

It is apparent that this study has not demonstrated the actual time of physiologic closure of the ductus, but it has shown that in some cases the ductus may remain open much longer than has previously been supposed. The fact that the veno-arterial shunt persists in some infants for several days indicates that the fall of pulmonary artery pressure from neonatal levels is variable and may depend upon other factors besides inspired oxygen tension.

3. The difference in oxygen saturation between blood samples from the upper and lower extremities in the newborn has another very practical implication. Many studies have been made by various workers in an effort to study the arterial oxygen saturation of the newborn. In many of these studies foot or umbilical artery blood has been used (34–37). In one of these studies (38) conclusions were drawn as to the chemical control of respiration in the newborn infant. The oxygen saturations thus obtained, in many instances, may have been low because of the veno-arterial shunt at the ductus, as demonstrated by the data in this paper. Values for carbon dioxide content and pH in the foot blood would also be different from true arterial blood by virtue of this venous admixture. Several studies using oximeters placed on the ears (39, 40) or foreheads (41) of newborn infants have thus avoided this venous admixture from the ductus and have shown arterial saturations comparable to those found in the right hands of the infants in this study. It is apparent therefore that in order to obtain true arterial blood from the newborn and to avoid the venous admixture due to the ductus, samples should be obtained from either the right upper extremity or the right ear lobe.

4. The absence of heart murmurs in these infants with blood flow through the ductus is of interest. Only one of the 43 newborn infants had a murmur, which was systolic in time. This absence of murmurs is comparable to the findings of Lyon, Rauh, and Stirling (42), who found murmurs in only a small percentage of newborn infants. A few of their cases showed at 24 hours of age a murmur which disappeared in a few days. We can make the same conclusion that they did, "that the remnants of fetal circulation rarely produce cardiac murmurs."

SUMMARY

1. Hand-foot oxygen saturations have been determined in 60 infants from 40 minutes to 11 months after birth. In a majority of infants under three hours of age, and in many infants under three days of age, the foot saturations are significantly lower than hand saturations.

2. Hypoxia will increase or re-establish the hand-foot oxygen saturation difference in infants up to 15 hours of age who have minimal initial hand-foot differences.

3. The following implications have been drawn from these studies:

(a) The ductus arteriosus may be open in the newborn human infant.

(b) Under these circumstances it is the site of a veno-arterial shunt of considerable magnitude which persists in a significant number of infants up to 72 hours of life.

(c) A high pulmonary artery pressure is present at birth and persists as long as the veno-arterial ductal shunt exists.

(d) The cessation of the veno-arterial ductal shunt is probably due to a fall in pulmonary artery pressure below the pressure in the aorta, rather than to an actual closure of the ductus.

(e) Studies of newborn blood gases, in which heel blood has been used, are not representative of true arterial blood since an admixture of venous blood from the ductus may be present.

(f) Heart murmurs are rarely heard in the newborn in spite of the early physiologic patency of the ductus arteriosus.

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THE PARTICIPATION OF BONE IN TOTAL BODY SODIUM METABOLISM IN THE RAT[1]

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(Submitted for publication November 15, 1954; accepted March 9, 1955)

Sixty years ago Gabriel demonstrated the presence of substantial amounts of sodium in the chloride-free residue of bone which had been extracted with alkaline glycol solutions (1). In 1936, Harrison, Darrow, and Yannet (2) defined "extra" bone sodium as that present in excess of the amount predictable from chloride content and suggested that at least 25 per cent of the total body sodium content might be bound chemically to bone salt. Subsequently, Kaltreider, Meneely, Allen, and Bale (3), Forbes and Perley (4), Stern, Cole, Bass, and Overman (5), and Edelman, James, Baden, and Moore (6) have shown that 30 to 40 per cent of bone sodium is exchangeable with circulating radiosodium within 24 hours. These workers have confirmed the earlier impressions of Harrison, Darrow, and Yannet by finding approximately one-third of total body sodium to be in the skeleton.

Reduction of bone sodium during acute acidosis has been demonstrated in dogs by Nichols and Nichols (7) and in rats by Bergstrom and Wallace (8). The former found an average decrease of 18.6 mEq. of sodium per kilogram of wet fat-free bone in dogs after four hours of sodium depletion by means of hemodialysis. Rats depleted of sodium and made acidotic by intraperitoneal dialysis against ammonium chloride solutions lost 42 mEq. of sodium per kilogram of wet bone within 48 hours. This represents 28 per cent of the total extra bone sodium and corresponds closely in magnitude to the fraction shown previously to be exchangeable with circulating radioisotope.

Darrow, Schwartz, Iannucci, and Coville (9), Cotlove, Holliday, Schwartz, and Wallace (10), and others have indicated that intracellular sodium may undergo wide fluctuations around its normal value of 9 to 10 mEq. per liter of muscle cell water. Concentrations of 1 mEq. per liter in acidosis and of 22 mEq. per liter in alkalosis are reported. Apparently sodium exists in the body in at least three phases: extracellular fluid, intracellular fluid, and bone salt. All participate in sodium metabolism, though bone sodium may consist of accessible and inaccessible fractions.

The work to be reported here concerns the following: 1) What proportion of total sodium loss in acute acidosis and sodium depletion is borne by bone sodium? 2) Is bone sodium content diminished by acidosis without large loss of sodium, *i.e.*, can changes in body fluid pH affect the pattern of bone electrolyte? 3) Can the fraction of bone sodium removed be increased by repeated depletion procedures? 4) During recovery from acidosis, what proportion of the sodium retained is deposited in the skeleton?

EXPERIMENTAL PROCEDURE

Six groups of adult male albino rats were used.

Group 1 (20 animals). Control rats were maintained on a diet of dog chow and water *ad lib.* At the time of sacrifice samples of bone and blood were taken for analysis as described below. Fourteen carcasses were then dried at 105°C., defatted, and analyzed for sodium, calcium, and chloride.

Group 2 (7 animals). These were depleted of sodium and made acidotic by transperitoneal dialysis against ammonium chloride. Each rat received intraperitoneally 10 per cent of its body weight of a solution containing 90 millimoles of ammonium chloride and 50 grams of glucose per liter. After a four-hour equilibration period the peritoneal fluid was withdrawn and analyzed for sodium. Approximately 25 per cent of the estimated total body sodium content is removed by this procedure (8). The animals were then allowed water but fasted for 48 hours prior to sacrifice. The total urinary output of four animals was collected during the 48-hour post-dialysis period and analyzed for sodium. The sampling procedure was that used for Group 1.

Group 3 (12 animals). After a 12-day control period on chow and water, eight animals in this group were

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[2] Markle Scholar in Medical Science.

given acetazoleamide (Diamox®)[3] in a concentration of 666 mg. per liter in their drinking water. The remaining four animals were continued on tap water. All were weighed daily throughout the experiment. Serum pH was measured in both control and experimental rats before and during treatment. After sixteen days of drug administration the rats were killed and sampled as above.

Group 4 (6 animals). These were given acetazoleamide as in Group 3. After 13 days of treatment they were subjected to dialysis as in Group 2. A 48-hour period of fasting preceded sampling. Blood and bone samples only were analyzed in this group.

Group 5 (7 animals). These were subjected to the same depletion procedure as Group 2. At the conclusion of the 48-hour fast they received 200 ml. per kilo of body weight of a solution containing 150 mEq. of Na, 100 mEq. of Cl, and 50 mEq. of HCO_3^- per liter. This was given intraperitoneally in two doses ten hours apart. After 24 hours of "repair" (72 hours after dialysis) the rats were killed and sampled.

Group 6 (9 animals). These were depleted as in Group 2. After the 48-hour post-dialysis period they were allowed chow and water *ad lib.* They were sacrificed five days later when they had regained 86 per cent of the weight lost after dialysis.

CHEMICAL METHODS

A. *Serum:* 1) Water was determined by the use of micropycnometers (10). 2) Sodium was measured by internal standard flame photometry (11). 3) Chloride was measured by the iodometric method of Van Slyke and Hiller (12). 4) Serum pH was determined at 38°C. with the bicolor standards of Hastings and Sendroy (13).

B. *Carcass:* 1) The method of preparation was that of Schwartz, Cohen, and Wallace (14), except that the animals were not exsanguinated; only small samples (2 to 3 ml.) of blood were taken. 2) Total body water was measured as weight lost after drying to constant weight at 105°C. 3) Fat was determined by extracting the dry material with several changes of a mixture of ethyl and petroleum ether and re-weighing. 4) Chloride was measured by back titration of excess silver after the dry fat-free material had been soaked for 24 hours in a measured excess of silver nitrate and dissolved and digested in nitric acid (14). 5) Calcium was precipitated as oxalate which was then titrated with permanganate. 6) Sodium was measured by internal standard flame photometry after removal of calcium by oxalate precipitation. Three successive recrystallizations of calcium oxalate were carried out in order to recover co-precipitated and adsorbed sodium (8).

C. *Bone:* The method of analysis has been presented in detail elsewhere (8). Samples included all of the long bones in most cases. In some instances only femora and tibiae were taken for analysis. No difference in results was seen. 1) *Water* was measured as weight loss after drying at 105°C. The bones were then ground to a fine powder and aliquots taken for subsequent analyses. 2) *Chlorides* were measured by an adaptation of Sunderman and Williams' method (15) to semi-micro quantities. 3) *Sodium* was determined by internal standard flame photometry on aliquots ashed and dissolved in nitric acid. Calcium separation was carried out as described above prior to flaming. 4) *Calcium* was titrated as oxalate with permanganate.

[3] This drug was kindly supplied by Drs. T. H. Maren and J. M. Ruegsegger of Lederle Laboratories.

CALCULATIONS

The concentrations of sodium and chloride in extracellular water were calculated from measurements of serum sodium, chloride, and water using Donnan's factors ($Cl_{ecw} = 1.02 \times Cl_{serum\ H_2O}$; $Na_{ecw} = 0.95 \times Na_{serum\ H_2O}$). Bone chloride was multiplied by the ratio Na_{ecw}/Cl_{ecw} to give the amount of bone sodium predictable from extracellular water content as defined by chloride. This was subtracted from total bone sodium (Na_t) to give "extra" bone sodium (Na_e) as originally defined by Harrison, Darrow, and Yannet (2). The ratio of extra bone sodium to calcium was then calculated. Total body calcium content was multiplied by the ratio Na_e/Ca to give the extra bone sodium content of the whole body. The assumption was made that the amount of non-skeletal calcium measured in total calcium was negligible.

The volume of distribution of extracellular sodium, (H_2O_{Na}), was derived by subtracting intracellular and bone sodium from total sodium content and dividing the remainder by the sodium concentration of extracellular water. Intracellular sodium was approximated by using the data of Cotlove, Holliday, Schwartz, and Wallace for muscle sodium in normal and acidotic rats (10), assuming a muscle mass equal to 40 per cent of body weight. Neglecting intracellular Na in tissues other than muscle introduces a source of error which makes the value for extracellular Na an estimate rather than a precise quantity.

The concentrations of chloride in red cell water and in collagen water differ from that in extracellular water, and the necessity of correcting the apparent chloride space for these two factors has been stated by Harrison, Darrow, and Yannet (2) and by Nichols, Nichols, Weil, and Wallace (16). According to Bernstein (17), the chloride concentration of rat erythrocyte water is 82 mEq. per L. If blood volume is assumed to be 10 per cent of body weight, hematocrit 50 per cent, and red cell water 720 ml. per Kg. of cells, erythrocyte chloride equals $0.100 \times .50 \times .72 \times 82 = 3$ mEq. per Kg. body weight. Nichols, Nichols, Weil, and Wallace (16), using the total body collagen data of Lightfoot and Coolidge (18), and analyses of tendon for water and collagen, calculated that 77 ml. of water per Kg. of body weight is associated with collagen and elastin. In this phase they found an average chloride concentration of 129 mEq. per L., which is 12 mEq. per L. greater than that calculated for extracellular water not so associated. The correction introduced amounts to $0.077 \times 12 = 0.9$ mEq. per Kg. body weight. The sum of both corrections (3 plus $0.9 = 3.9$)

TABLE I

Average changes following intraperitoneal dialysis (seven rats)

Body Weight			Sodium		
Before (Gm.)	After (Gm.)	% Loss	Original mEq/Kg.	Removed mEq/Kg.	Removed %
246	197	20	52.8	11.4	21.6

was subtracted from total chloride content before calculating the volume of distribution.

RESULTS

1. *Acute sodium depletion and acidosis*

Table I shows the average weight loss following dialysis in Group 2 and the amount of sodium removed as determined by analysis of the dialysate. Sodium withdrawn is expressed as per cent of total body content, calculated from original body weight and average normal sodium content (Table II). The total urinary sodium output during 48 hours was less than 1 mEq. per kilo of body weight per day. There was no change in serum sodium concentration or in sodium content in mEq. per kilo of final body weight (Table II). The ratio of sodium to calcium in bone was 0.016 as compared to 0.021 in control animals (Table III).

From body composition after dialysis and per cent of weight lost it is possible to calculate the amount of sodium lost per kilogram of original body weight. Table IV shows the total loss so calculated and the contribution of each phase. The results are shown as mEq. per Kg. before and after dialysis and as mEq. per 800 gm. The latter notation indicates the loss sustained by each original Kg. after a 20 per cent weight loss. The average total extra bone sodium content of 14 normal rats was 11.2 mEq. per Kg. fat-free wet weight (Total calcium = 546 mEq. per Kg.; Na_e/Ca ratio = .0206). In seven dialyzed rats total calcium was 633 mEq. per Kg. and Na_e/Ca was .0163. Total extra bone sodium was therefore 10.3 mEq. per Kg. Since body weight after dialysis was 0.8 × original weight (Table I) the decrease in extra bone sodium was $11.2 - (10.3 \times .8) = 3.0$ mEq. per original Kg. This represents 28 per cent of the total sodium loss of 10.8 mEq. per Kg.

Values for the intracellular phase were calculated from the muscle data of Cotlove, Holliday, Schwartz, and Wallace for rats subjected to an identical depletion procedure (10). These workers found that the intracellular sodium content of muscle after dialysis was 0.6 mEq. per Kg. of muscle as compared to 6.0 mEq. per Kg. in con-

TABLE II

Body composition in control, dialyzed, and repaired rats

Group (#)	Na		Ca	Cl		H_2O_{Na}	H_2O_{Cl}	H_2O_T
	mEq/Kg fat-free wet wt.	mEq/L ECW	mEq/Kg. fat-free wet wt.	mEq/Kg fat-free wet wt.	mEq/L ECW	ml/Kg fat-free wet wt.		
1. (Control) (14)	53	155	546	36	114	251	282	732
2. (Dialyzed) (7)	53	153	633	39	107	274	329	721
3. (Diamox) (6)	51	153	559	37	111	—	298	721
5. (Repair) (7)	59	150	621	42	97	—	393	731

TABLE III

Bone composition

Group (#)	mEq/Kg. wet weight			
	Na_t	Na_c	Ca	Na/Ca
1. Control (20) (S.E.)	198	161	7900	.0206 (.0007)
2. Dialysed (7) (S.E.)	162	124	7600	.0163 (.0012)
3. Acetazole Amide (7)	152	120	8000	.0150
4. Acetazole amid plus Dialysis (6)	176	138	7900	.0174
5. Parenteral Repair (7) (S.E.)	143	98	7200	.0136 (.0008)
6. Oral Repair (9) (S.E.)	164	136	7700	.0175 (.0005)

trol rats. If muscle mass is assumed to equal 40 per cent of body weight originally, intracellular sodium loss per Kg. will equal $.4 \times (6.0 - 0.6) = 2.2$ mEq. The error introduced by decrease in muscle mass during weight loss will be small since the intracellular sodium of post-dialysis muscle is minimal. The loss sustained by extracellular fluid may be estimated as total Na loss—(bone loss + intracellular loss).

The total loss by analysis, 10.7 mEq. per original kilogram, is 20.5 per cent of the original content, a figure which agrees well with the measured removal of 22 per cent indicated in Table I. Fifty-two per cent of the total loss was sustained by extracellular sodium, 28 per cent by bone sodium, and 20 per cent by intracellular sodium.

2. Acetazoleamide treatment

Figure 1 shows the changes in body weight, growth rate, and serum pH observed during the

TABLE IV

Distribution of sodium in control and depleted rats

Phase	Control	Depleted		mEq/Kg. Loss	% of Total Loss
	mEq/Kg fat-free		mEq/800 gm.		
Total	52.8	52.5	42.0	10.8	100
Na_{ic}*	2.4	0.3	0.2	2.2	20
Na_b	11.2	10.3	8.2	3.0	28
Na_{ec}	39.1	41.9	33.5	5.6	52

*Calculated from data of Cotlove et al.

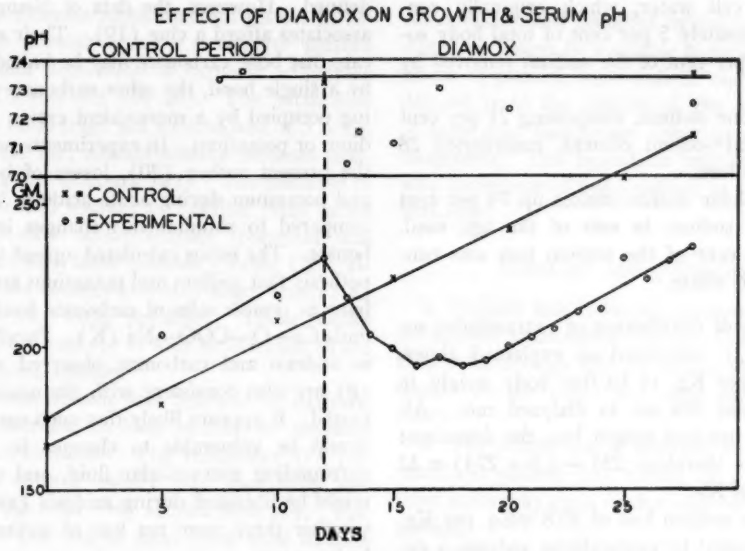

FIGURE 1

administration of acetazoleamide (Diamox®). After an initial weight loss, growth rate equalled that of the controls, although serum pH was depressed throughout the experimental period. Total body composition (Table II) did not differ significantly from that of control animals. Bone sodium to calcium ratio (Table III) was .0150, or 71 per cent of the normal value of .0206 ($p = .01$).

3. *Acetazoleamide plus dialysis*

Dialysis of rats pre-treated with acetazoleamide for 13 days (Group 4) resulted in the removal of 29 per cent of calculated total body sodium content. Bone analysis after 48 hours showed a sodium to calcium ratio of .0174 as compared to .0163 for rats dialyzed without pre-treatment and .0150 for rats treated with acetazoleamide without dialysis. There was apparently no further loss of bone sodium following dialysis in rats whose bone sodium to calcium ratio had previously been reduced by acetazoleamide.

4. *Parenteral repair*

Table II shows the body composition of rats treated with parenteral sodium-containing fluid after dialysis. Total sodium was significantly increased (59 mEq. per Kg.) over that found following dialysis and in normal controls. None of the sodium increment could be assigned to the skeletal phase, since bone sodium to calcium ratio was .0136. This is lower than the ratio found in depleted animals (.0163) but the difference is not statistically significant ($p = .05$).

5. *Oral repair*

After five days of recovery on a normal diet, the rats in Group 6 had regained 86 per cent of their post-dialysis weight loss and were growing at the same rate as controls. Their bone composition (Table III) was not significantly different from that of Group 2 animals sacrificed immediately after depletion. There was apparently no sequestration of sodium in bone during the recovery period.

DISCUSSION

Intraperitoneal dialysis against ammonium chloride with subsequent removal of peritoneal fluid causes both sodium depletion and acidosis. Significant decreases in serum pH and CO_2 content have been previously reported (8). Three phases contributed to the total sodium loss (Table IV):

A. Muscle cell water, which originally contained approximately 5 per cent of total body sodium, lost 20 per cent of the sodium removed by dialysis.

B. Extra bone sodium, comprising 21 per cent of normal total sodium content, contributed 28 per cent of the loss.

C. Extracellular sodium makes up 74 per cent of total body sodium in rats of the age used. Fifty-two per cent of the sodium loss was contributed by this phase.

The volume of distribution of extracellular sodium, (H_2O_{Na}), calculated as explained above, was 251 ml. per Kg. of fat-free body weight in control rats and 274 ml. in dialyzed rats. Allowing for 20 per cent weight loss, the decrement in H_2O_{Na} was therefore $251 - (.8 \times 274) = 32$ ml. per original Kg.

If the entire sodium loss of 10.8 mEq. per Kg. had been sustained by extracellular sodium, a decrease of approximately 60 ml. or 24 per cent would have been necessary to preserve normal sodium concentration in extracellular fluid following dialysis.

The volume of distribution of chloride (H_2O_{Cl}, corrected for red cell and collagen water as explained above) is compared with H_2O_{Na} in Table II. H_2O_{Cl} exceeds H_2O_{Na} in both normal and dialyzed animals. Since the methods for estimating both phases are indirect and involve several steps (see "Calculations" above), no significance can be assigned to the difference. The discrepancy is greater in the dialyzed animals, which may be due to the fact that dialysis against ammonium chloride caused a greater loss of sodium than of chloride.

Weight loss (20 per cent) and sodium loss (21 per cent) were nearly identical after dialysis. This implies a proportionate reduction of body mass during the 48-hour post-dialysis fast. Since the composition of weight loss during fasting varies with the length of the fast, the close agreement between loss of sodium and loss of weight seen in these experiments must be coincidental.

Since both acidosis and sodium depletion followed dialysis, the relative importance of each in bone sodium mobilization is not indicated. The chemical relationship of sodium to the apatite crystal structure of bone has not been precisely defined. However, the data of Neuman and his associates afford a clue (19). Their studies indicate that bone carbonate may be linked to calcium by a single bond, the other carbonate valence being occupied by a monovalent cation such as sodium or potassium. In experiments performed by the present author (20), losses of bone sodium and potassium during acute acidosis in rats were compared to simultaneous changes in bone carbonate. The ratios calculated agreed with the hypothesis that sodium and potassium are present in bone as double salts of carbonate having the formula Ca—O—COO—Na (K). Parallel increases in sodium and carbonate observed with ageing (8) are also consistent with the association suggested. It appears likely that such carbonate salts would be vulnerable to changes in pH of the surrounding extracellular fluid, and that sodium might be liberated during acidosis (as $NaHCO_3$) whether there were net loss of sodium from the body or not.

The work with Na^{24} mentioned above (3–6) has shown that a fraction of bone sodium (approximately 30 per cent) is in equilibrium with circulating radiosodium. Rapid dialysis might cause a shift of this fraction by reducing the sodium concentration of extracellular fluid. However, exchangeable sodium is apparently entirely removed by peritoneal dialysis against ammonium chloride, while the sodium concentration of extracellular fluid is reduced slightly if at all (8, 10). This suggests that the concomitant acidosis may be of crucial importance in the mobilization of bone sodium.

The imposition of direct acid loads in the rat has had varying results. Irving and Chute (21) found a reduction in bone carbonate following acute oral HCl loads. Burns (22) reported that prolonged feeding of 0.1 normal HCl (12 to 100 ml. per rat per day) had no effect on bone carbonate. Neither of these studies included measurements of serum pH. Ingalls, Donaldson, and Albright (23) observed demineralization of bone in nephrectomized rats given HCl, but their report includes no chemical analyses.

Acetazoleamide, (Diamox®), a substituted sulfonamide, is known to inhibit carbonic anhydrase activity. It increases urinary excretion of sodium and potassium, presumably by interfering with tubular exchange of hydrogen for other

cations. Continuous administration results in return of the normal urinary electrolyte pattern with persistent depression of carbonic anhydrase activity and serum pH (24–26). The initial diuresis causes an average net sodium loss of 5 mEq. per Kg. in the rat; sodium balance becomes positive within 24 to 48 hours (26). The decrease in bone sodium and sodium to calcium ratio found in Group 3 after acetazoleamide treatment was equal to that seen after dialysis (Table III), although the net sodium loss was much less (5 as compared to 10.8 mEq. per kilo). These results suggest that acidosis *per se* may be essential to the removal of bone sodium.

When acetazoleamide was followed by dialysis, no further decrease in bone sodium or sodium/calcium ratio occurred. The remainder (71 per cent of the control value) was apparently not available to the total body sodium economy. The lability of 29 per cent of total extra bone sodium indicated here is in close agreement with previous findings discussed above.

The exchangeability of bone sodium with circulating radiosodium and its availability in acidosis and sodium depletion suggest the possibility of sequestration of this cation in bone during recovery from acidosis. Such storage would influence interpretation of sodium balance during treatment of diarrhea, diabetic acidosis, and other disorders involving electrolyte loss. Darrow (27) has shown that more sodium may be retained during therapy for diarrhea than can be accounted for by measured extracellular increments. Gamble and his associates have found similar results following sodium loads in normal infants (28). In the present study neither parenteral nor oral repair of depleted animals caused significant elevation of bone sodium (Table III). In the former instance an increment of total body sodium was measured; in the latter it was inferred from recovery of the weight lost during depletion (Table II). Apparently little or none of the sodium retention observed during recovery from acidosis can be assigned to the skeletal phase. It has been mentioned above that bone sodium is probably mobilized according to the equation Ca—O—C—OO—Na + H-Anion → Ca-Anion + $NaHCO_3$. The anion involved has not been identified. The composition of bone salt and body fluid both suggest phosphate as a possibility. If so, the calcium valences formerly occupied by —O—C—OO—Na would no longer be available to sodium, and restitution of normal bone sodium content would be delayed until the surface of the apatite crystals of bone had undergone either growth or re-arrangement. This hypothesis may explain the one-way movement of bone sodium in relatively acute situations.

SUMMARY AND CONCLUSIONS

1. Sodium loss following intraperitoneal dialysis against ammonium chloride is shared by extracellular sodium (52 per cent), intracellular sodium (20 per cent) and bone sodium (28 per cent).

2. The production of acidosis without large or sustained sodium loss results in a decrement of bone sodium as great as that seen after sodium depletion.

3. Only 29 per cent of bone sodium content can be mobilized by any procedure studied thus far.

4. No sodium is deposited in the skeleton of the rat following either oral or parenteral repair of acidosis and sodium depletion within the period studied (five days).

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THE MECHANICAL PROPERTIES OF THE LUNGS IN EMPHYSEMA[1]

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In pulmonary emphysema normal respiratory mechanisms become distorted almost beyond recognition, and familiar landmarks such as "dead space," "alveolar gas" and "effective ventilation" virtually disappear. At the same time, these very distortions have served as a stimulus for studies which have led to a greater understanding of the problems of distribution of ventilation, and of blood flow within the lungs.

In attempting to study the mechanics of breathing in emphysema, the authors have also found that concepts based on the behavior of normal lungs are inadequate to account for their observations. This has led to a consideration of the influence of local mechanical factors on the over-all mechanical properties of the lungs. Since these considerations are also pertinent to problems of the distribution of gas flow within the lungs, and possibly also to the pathogenesis of emphysema, they will be emphasized in this report. The details of the theoretical treatment will appear elsewhere.

METHODS

Ten patients with advanced pulmonary emphysema and ten normal individuals have been studied. Details of the methods have been reported elsewhere (1).

Methods of measurement

Rates of flow were recorded at the mouth with a Silverman pneumotachograph screen connected to a manometer. Volume changes were measured by electrical integration of the rate of flow signal coming from the pneumotachograph manometer. This method of recording rates of gas flow and volume changes has the advantages of excellent response characteristics and of imposing a negligible external resistance. It has the disadvantage that volume changes occurring over a period greater than fifteen seconds cannot be recorded accurately because of the limitations imposed by the integrator. For such slow volume changes a recording spirometer was used.

As a measure of transpulmonary pressures the pressure difference between the mouth and a point within the lower third of the esophagus was recorded. The intra-esophageal pressure was transmitted to one side of a differential manometer by means of an air-filled polyethylene tube 64 cm. in length, 1 mm. in internal diameter, with eight to ten spirally arranged holes in the catheter wall at the distal end. A thin-walled rubber balloon with a volume of 1.5 ml. undistended and 3.2 cm. in length was sealed to the catheter and enclosed the openings. The other side of the manometer membrane was connected to the mouthpiece chamber by pressure tubing. The catheter was passed through the nose and was readily swallowed. The air volume of the balloon during recording was *0.5 to 1.0 ml.* The flow rates, volumes and pressures were recorded simultaneously on a direct-writing oscillograph.

The pressure difference existing at any instant between the mouth and the surface of the lungs depends on the elastic recoil of the lungs and the flow-resistance of the airways and lung tissue. The elastic component of pressure increases as the lungs are inflated, and the relationship of lung volume change to the corresponding elastic pressure change serves as a measure of the lungs' elastic properties. The flow-resistive component of pressure depends on the *rate* at which the lungs are *changing* volume, and the relationship of this component to the simultaneous rate of volume change of the lungs serves as a measure of the lung and airway flow-resistive properties.

In analyzing the simultaneous recordings of volumes, rates of flow, and pressures in order to obtain information about the mechanical properties of the lungs, the principal step is the separation of the total pressure into its elastic and flow-resistive components. This separation may be obtained by graphic means from measurements of the Poly-Viso tracing.[2] This tedious procedure may be avoided, however, by using a cathode-ray oscilloscope to plot the variables.

Two types of patterns were produced on the oscilloscope face. In one (Figure 1A) volume changes appeared on the "Y" axis and the mouth-to-esophagus pressure difference on the "X" axis. In this instance the respiratory cycles appeared as loops. At the instants of flow reversal at the "top" and "bottom" of the loops the flow-resistive component of pressure was zero and only the elastic component remained. The slope of a line

[1] Aided by a research grant from the National Tuberculosis Association.

[2] Sanborn Company, Cambridge, Massachusetts.

drawn through the volume extremes of a loop therefore expressed the ratio of volume change to elastic pressure change. Expressed in liters per cm. of water this slope defined the *compliance* of the lungs for that particular breath. (The inverse of *compliance* is *elastance*).

A second pattern was used to measure the flow-resistance of the lungs (Figure 1B). In this instance the rate of air flow appeared on the "Y" axis and the flow-resistive component on the "X" axis. The latter quantity was obtained by first registering the total mouth-to-esophagus pressure difference on the "X" axis as before. Again a loop was produced with each respiratory cycle. By then applying the volume variation to the "X" axis so as to be subtracted electrically from the total pressure variation, the loop could be caused to "close" at the "X" axis. In effect, this subtracted from the total pressure variation just the elastic component, and the remainder represented the flow-resistive component.[3] The pattern then produced showed the relationship between the rate of flow and the pressure producing this flow at every instant during the respiratory cycle. The flow-resistance at any instant was expressed by the ratio of pressure to simultaneous flow rate in cm. of water per liter per second.

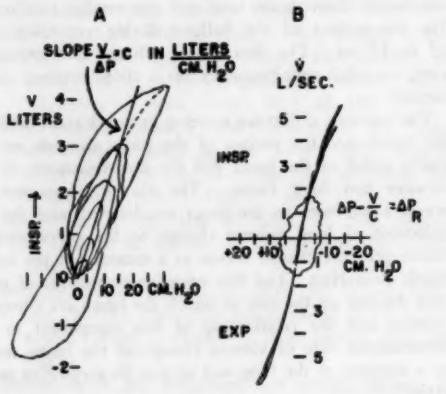


FIG. 1. EXAMPLES OF THE TWO TYPES OF PATTERNS OBTAINED WITH THE OSCILLOSCOPE

For a description of these patterns see the text.

[3] This procedure is based on the following relationship. The total pressure is the algebraic sum of its elastic and flow resistance components. In the range of the resting tidal volume the elastic component is linearly related to change in volume (1–3). Therefore the change in volume multiplied by the proper constant equals the elastic component. The volume gain control on the "X" axis represents this constant. By adjusting this control various constants can be selected. The proper constant is obtained when the loop is closed. This follows from the fact that the flow-resistive pressure must be zero when flow is zero. Thus, as the flow passes through zero, it must intersect the "X" axis at the same point both at end inspiration and end expiration, closing the loop at the "X" axis.

Tracings of the oscilloscope patterns were obtained in pencil by means of an indirect viewer.[4] In instances where the "fatness" of the pressure-volume loops made accurate measurement of the points of minimum and maximum volume impossible, measurements were made directly from the Poly-Viso tracings obtained at high paper speeds (50 mm. per second).

Experimental procedure

Subjects were studied in the sitting position. The balloon was passed to the lower third of the esophagus. A nose clip was applied and the subject was instructed to breathe with the lips held firmly around the mouthpiece (2.5 cm. internal diameter).

Tracings were obtained during quiet breathing and during a variety of specific maneuvers which will be described with the results.

Tables I and II present pertinent clinical findings and the results of a number of conventional pulmonary function studies for the group of patients. Table III describes the normal group. The age range of the ten normal men was from 32 to 57; the average age was ten years less than that of the patient group.

Clinically, the patients represented a fairly homogeneous group. Their ages ranged from 29 to 69 years and all were men. Six patients had either a long history of bronchial asthma or repeated pulmonary infections; bronchiectasis was present in one. Dyspnea was always the chief complaint; in about one-half it was present at rest, and the other patients were short of breath on slightest exertion. Physical examination, chest roentgenograms and fluoroscopic observation invariably showed evidence of severe pulmonary emphysema. None of the patients was considered to have cor pulmonale, and none had an enlarged liver, ascites, or edema. The results of the conventional pulmonary function studies were consistent with the diagnoses of severe pulmonary emphysema in every instance.

RESULTS

Quiet breathing

Figure 2 shows a typical tracing of the mouth-to-esophagus pressure difference, rate of flow, and volume change during quiet breathing in a patient with emphysema. A similar tracing from a normal subject is shown for comparison.

Although the tidal volumes and breathing frequencies in the two instances are nearly the same, the breathing patterns are strikingly different. Prolonged expirations and obliteration of coarse irregularities in the flow patterns were common

[4] Oscillotracer, Robert A. Waters, Inc., Waltham, Massachusetts.

TABLE I

Clinical findings in ten patients with emphysema

Patient	Probable etiology	Duration complaints	Dyspnea	Complications and diseases other than etiologic	Past treatment	Cyanosis	Hematocrit	Clubbing	Chest X-ray and fluoroscopy
		yrs.							
Wh	Influenza 1918 Pneumonia 4 yrs. ago	4	++++	None	Digitalis Expectorants	0	—	0	Flat diaphragm Increased basal markings
Su	Hay fever, Allergic bronchitis	15	++ to +++	None	Allergen extracts	±	52	0	Increased lung measurements Low, flat diaphragm
Mi	Bronchial asthma	25	++++	Bronchiectasis, both lower lobes	Antibiotics Bronchodilators	+	41	+	Fixed low diaphragm Peribronchial infiltration
Sh	Unknown	4	+++	Spontaneous pneumothorax	Pneumoperitoneum Expectorants	±	—	0	Flat, low diaphragm Trapping
Br	Asthma Chronic bronchitis	31	+++	Chronic mastoiditis	Antibiotics Postural drainage	+	50	+	
Pi	Chronic bronchitis Asthma	15	++++	Respiratory acidosis Transient atelectasis	Bronchodilators	+	61	0	
Mc	Unknown	5	+++	None	Pneumoperitoneum	0	50	0	Flat, low diaphragm; marked trapping
Py	Foundry worker Bronchial asthma	1	+++	None	Antibiotics Antihistaminics	0	50	0	Blunted costophrenic angles Marked trapping
Wi	Unknown	5	+++	None	Antibiotics Bronchodilators	+	56	0	Increased marking Low, fixed, flat diaphragm
Bo	Onset after pneumonia, ? Allergy	3	++++	None	Antibiotics Bronchodilators	+	45	0	Concave diaphragm Paradoxical motion

TABLE II

Lung volumes, ventilation and intrapulmonary mixing

Patient	Sex	Age	B.S.A.	M.B.C.*	Air velocity index	Exercise ventilation*	Exercise dyspnea index	Resting ventilation*	Ventilation equivalent for oxygen	Vital capacity*	Residual volume*	RV/TLC X100	Alveolar N_2 after 7' O_2	Timed capacities % of total vital capacity		
		yrs.	*m²*	*L./min.*		*L./min.*	*%*	*L./min./m² BSA*		*cc.*	*cc.*	*%*		*1 sec.*	*2 sec.*	*3 sec.*
Wh	M	69	1.79	21	0.48	Unable	—	4.27	3.2	1,950	3,960	67	5.5	30	42	54
Su	M	60	2.06	37	0.35	29	78	5.49	2.4	3,850	3,800	50	9.7	23	49	57
Mi	M	29	1.67	24	0.51	10	41	4.18	2.3	1,880	4,180	69	8.4	30	51	65
Sh	M	69	1.73	36	0.49	26	72	5.89	4.0	2,840	3,970	58	7.2	24	39	48
Br	M	32	1.94	48	0.46	20	41	5.32	2.4	3,190	3,280	51	8.9	31	47	67
Mc	M	59	1.73	31	0.59	18	58	4.28	3.5	2,370	3,860	62	6.7	30	47	63
Py	M	68	2.05	78	0.60	31	40	5.36	3.8	3,270	3,280	50	1.3	42	60	68
Wi	M	42	1.73	24	0.45	Unable	—	4.83	3.1	2,520	4,000	61	8.0	30	43	60
Bo	M	35	1.57	21	0.37	19	85	6.27	3.4	2,110	5,830	74	9.1	25	33	48
Predicted Normal				102	1.00	18	18	3.90	2.6	3,790	1,360	26	1.50	83	94	97

* BTPS.

TABLE III

Age, height, and body surface area of ten normals

Name	Age	Ht.	B.S.A.
	yrs.	*cm.*	m^2
Le	32	173	1.79
Co	54	162	1.44
Mo	55	163	1.63
Af	33	162	1.66
Go	34	183	2.04
Wh	38	187	2.12
Ca	57	179	1.89
Fa	40	166	1.74
Me	32	187	2.06
Fe	33	189	2.12

to all of the patients studied. The relatively large amplitude of the pressure variation was also characteristic.

Figure 3A shows the volume-pressure tracings for the cycles shown in Figure 2. The compliance of the lungs is about the same in the two cases. (The zero flow pressure at end-inspiration was obtained from the tracings, Figure 2.) The "fatness" of the loop is much greater in the patient with emphysema. This is due to the increased resistance and is shown more clearly in Figure 3B in which the flow-resistive pressure difference is on the abscissa and the rate of flow on the ordinate. In this graph inspiration and expiration both start and end at the origin. During inspiration the relationship of flow to pressure is the same for the increasing and decreasing phases of flow rate resulting in single curved lines. This is also the case during expiration in the normal, but the patient's expiration describes a loop. As stated above, pulmonary flow-resistance may be expressed as the pressure necessary to produce a given rate of flow. Early in expiration, the patient accomplishes a flow of 0.2 liters per second at the cost of a little more than 2 cm. of water pressure. Later, the same flow costs more than three times this pressure. This increase in expiratory resistance is plotted against time (Figure 3C). Here ratios of resistive pressure to flow are plotted at intervals during the respiratory cycle. At all times the pulmonary flow resistance of the patient is much greater than normal, and during expiration it becomes thirty times as great.

A summary of results for all of the patients and normal subjects during quiet breathing is shown in Table IV. The compliance values in all cases are the mean of at least five separate measurements. The resistance values represent the highest values measured during inspiration and expiration, corresponding to the "peaks" in Figure 3C.

The compliances of four of the patients are below the normal range, while only one lies above. The average compliance in the patients is somewhat less than in the normal individuals, suggesting that the patients' lungs tended to be somewhat

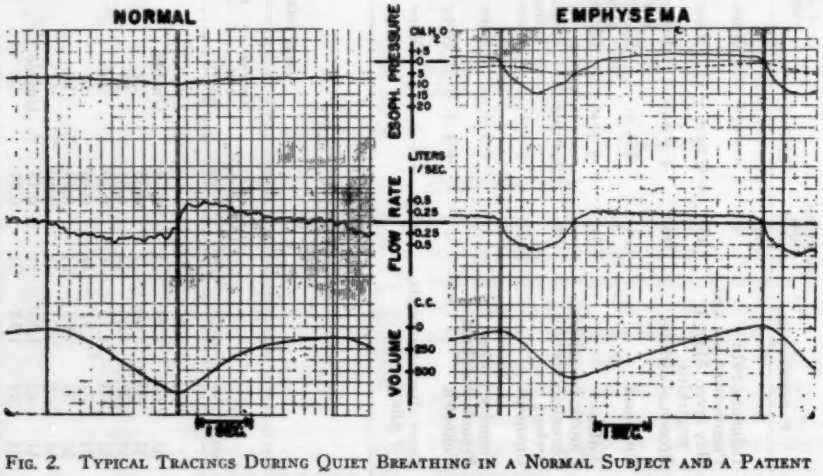


FIG. 2. TYPICAL TRACINGS DURING QUIET BREATHING IN A NORMAL SUBJECT AND A PATIENT WITH PULMONARY EMPHYSEMA

The dotted line on the pressure tracing on the right represents the calculated elastic component of pressure change.

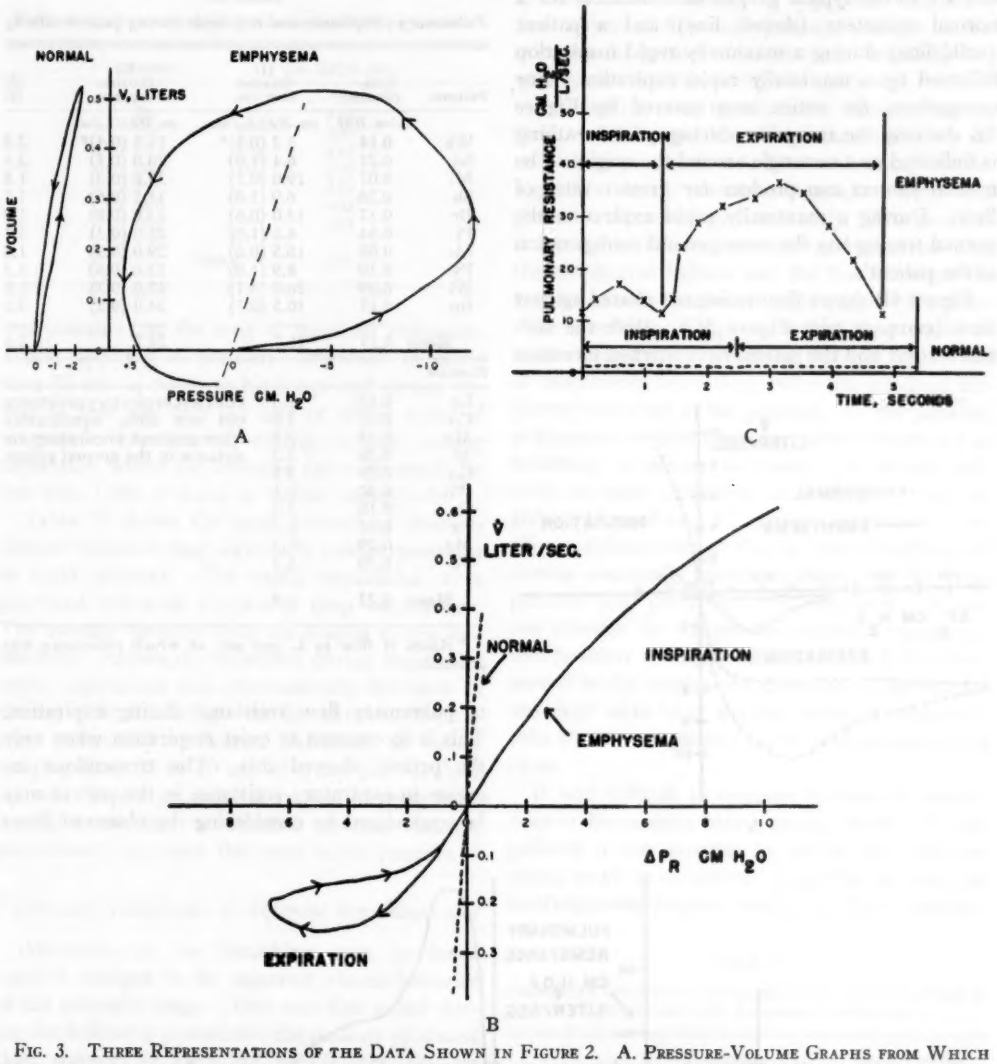


FIG. 3. THREE REPRESENTATIONS OF THE DATA SHOWN IN FIGURE 2. A. PRESSURE-VOLUME GRAPHS FROM WHICH PULMONARY COMPLIANCE MAY BE MEASURED. B. RESISTIVE PRESSURE-FLOW GRAPHS FROM WHICH PULMONARY FLOW RESISTANCE MAY BE MEASURED. C. GRAPHS OF PULMONARY FLOW RESISTANCE CHANGES DURING THE RESPIRATORY CYCLE

less compliant than normal during quiet breathing.

The differences in flow resistances between the groups are more marked. The patients have increased inspiratory flow resistance. No consistent difference in inspiratory and expiratory resistance was found in the normals. In contrast, the greatest expiratory resistance was invariably larger than the greatest inspiratory resistance in the patients.

Pulmonary resistance during voluntary hyperventilation

Greater differences between the two groups were brought out during hyperventilation. Fig-

ure 4A shows typical graphs of resistances for a normal volunteer (dotted line) and a patient (solid line) during a maximally rapid inspiration followed by a maximally rapid expiration. For comparison, the entire area covered by Figure 3B showing the resistance during quiet breathing is indicated as a rectangle around the origin. The normal subject can produce far greater rates of flow. During a maximally rapid expiration the normal tracing has the same general configuration as the patient's.

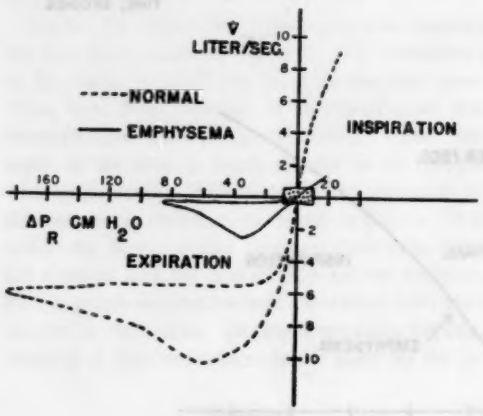
Figure 4B shows flow resistance plotted against time (compare with Figure 3C). Both the normal subject and the patient have marked increases of pulmonary flow resistance during expiration. This is in contrast to quiet respiration when only the patient showed this. The tremendous increase in expiratory resistance in the patient may be appreciated by considering the observed flows

TABLE IV

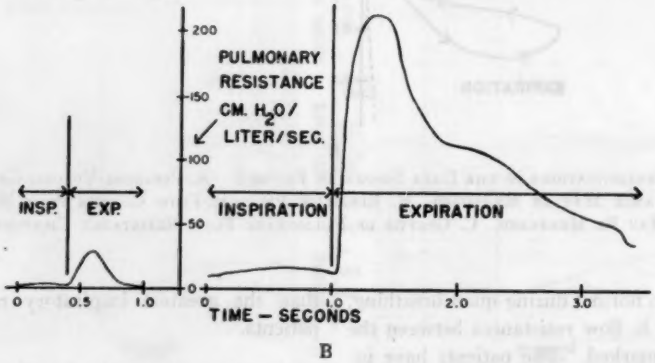
Pulmonary compliance and resistance during quiet breathing

Patients	Compliance	(1) Greatest insp. res.	(2) Greatest exp. res.	(2)/(1)
	L./cm. H_2O	*cm. H_2O/L./sec.*	*cm. H_2O/L./sec.*	
Wh	0.14	5.2 (0.8)*	14.5 (0.4)*	2.8
Su	0.21	6.4 (1.0)	20.0 (0.5)	3.1
Mi	0.07	19.0 (0.7)	35.0 (0.3)	1.8
Sh	0.26	6.9 (1.0)	11.5 (0.6)	1.7
Br	0.17	13.0 (0.6)	23.0 (0.8)	1.8
Pi	0.44	4.5 (1.0)	21.0 (0.3)	4.7
Mc	0.09	16.5 (0.6)	29.0 (0.3)	1.8
Py	0.10	8.9 (1.0)	12.0 (0.6)	1.3
Wi	0.09	26.0 (0.7)	47.0 (0.2)	1.8
Bo	0.17	10.5 (0.7)	34.0 (0.3)	3.2
Mean	0.17	11.7	24.7	2.4
Normals				
Le	0.15	2.3	Greatest expiratory resistance did not differ significantly from greatest inspiratory resistance in the normal group.	
Co	0.22	1.9		
Mo	0.15	2.9		
Af	0.20	1.2		
Go	0.26	2.8		
Wh	0.30	1.1		
Ca	0.16	2.4		
Fa	0.13	1.7		
Me	0.29	1.2		
Fe	0.30	1.2		
Mean	0.22	1.9		

* Rates of flow in L. per sec. at which resistance was greatest.



A



B

FIG. 4. A. RESISTIVE PRESSURE-FLOW GRAPHS DURING MAXIMALLY RAPID INSPIRATIONS AND EXPIRATIONS FOR A NORMAL (DOTTED LINE) AND A PATIENT (SOLID LINE). B. FLOW RESISTANCE CHANGES DURING THE COURSE OF MAXIMALLY RAPID INSPIRATIONS AND EXPIRATIONS FOR THE EXAMPLES SHOWN IN 4A. Left, normal; right, emphysema.

TABLE V

Pulmonary resistance during maximally rapid expirations

Patient	Cm. H_2O/L./sec.
Wh	98
Su	139
Sh	300
Pi	122
Mc	210
Py	330
Wi	300
Bo	480
Mean	247

and pressures at the time of maximal resistance. While applying a pressure difference of more than 80 cm. of water to his lungs and airway the rate of flow was only 0.5 liter or of the order of magnitude encountered during normal resting expiration where the resistive pressure would be less than 1 cm. of water in normal individuals.

Table V shows the peak pulmonary flow-resistance values during maximally rapid expirations in eight patients. The rapid expirations were produced following maximally deep inspirations. The average increase from the resting values was ten-fold. Pulmonary resistance during maximally rapid inspirations was approximately the same as during quiet breathing. In the normals, expiratory pulmonary resistance increased to values ranging from 10 to 30 cm. of water per liter per second. This represented a marked increase from resting levels, but the absolute increase was approximately one-tenth that seen in the patients.

Pulmonary compliance at different breathing rates

Alteration in the breathing rate produced marked changes in the apparent elastic behavior of the patient's lungs. This was first noted during the following procedure: the patients produced tidal volumes of from 0.2 to 1.0 liter, first as slowly as possible and then with increasing frequency up to maximally rapid rates. At high breathing frequencies the rate of change of intraesophageal pressure is high at the extremes of the tidal volume, and a valid measurement of the pressures at the instants of zero air flow is possible only if the pressure and flow recording systems are capable of reproducing precisely the timing and magnitude of the pressure and flow events. Although the response characteristics of the two systems were sufficient to reproduce accurately sine waves of flow and pressure change at frequencies far greater than the peak breathing frequency observed, an additional check was made on an artificial system in which the wave forms encountered in rapid breathing could be more accurately reproduced. A flow resistance, consisting of a small pneumotachograph screen was placed in the mouthpiece assembly. The pressure drop across this resistance was measured with the esophageal balloon and the flow through the resistance with the pneumotachograph. No phase differences between pressure and flow could be demonstrated while breathing through this system at frequencies more than twice the maximal frequency observed in the patients. In the patients, pulmonary compliance progressively decreased as breathing frequency increased. In normal subjects no such alterations of compliance are observed (4). Table VI presents pulmonary compliance values obtained during quiet breathing and during maximally rapid breathing for the eight patients who performed this maneuver. It was not possible to control the patients' respiratory mid-position during these efforts and it always moved in the inspiratory direction. The changes observed were of a greater order of magnitude than could be explained by the mid-position shifts alone.

It was difficult to measure pulmonary compliance at frequencies below resting levels. In two patients it was possible to extend the measurements down to conditions of no flow by using an interrupter technique similar to that described

TABLE VI

Pulmonary compliance during quiet and rapid breathing in eight patients with pulmonary emphysema

Patient	Quiet breathing		Rapid breathing	
	Resp. rate	Pulm. compliance	Resp. rate	Pulm. compliance
		liters/cm. H_2O		*liters/cm. H_2O*
Wh	21	0.14	75	0.06
Su	14	0.21	75	0.09
Mi	16	0.07	60	0.02
Sh	23	0.26	100	0.05
Br	18	0.17	135	0.02
Pi	21	0.44	150	0.03
Mc	17	0.09	60	0.03
Py	16	0.10	45	0.03
Mean		0.19		0.04

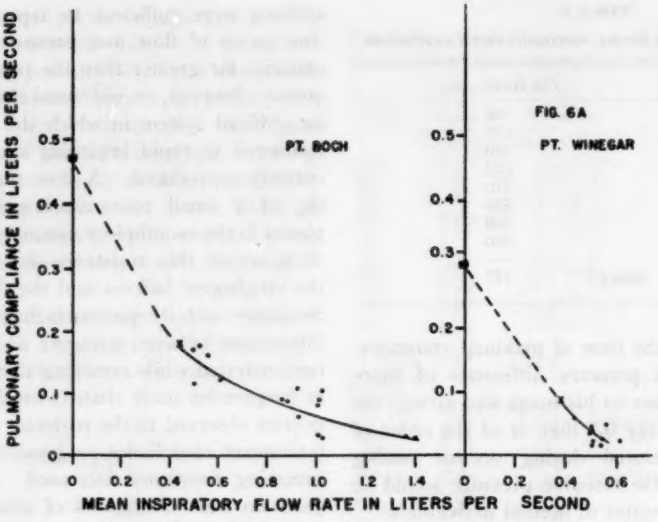


FIG. 5. PULMONARY COMPLIANCE IN TWO PATIENTS

The squares on the ordinate are "static" values. The X's indicate values obtained during quiet breathing and the dots, values obtained during voluntary hyperventilation.

by Stead, Fry, and Ebert (3). Mouth-to-esophageal pressure difference was measured following interruption of the air stream, in this case by means of a solenoid-driven valve in the mouthpiece assembly. The valve was triggered by a switching device attached to a spirometer from which the patient inspired. At volume increments of 0.25 liter the valve closed for pre-set time intervals. Pressure differences reached a steady level, except for the continuing oscillation synchronous with the cardiac cycle, in periods less than one second. Volume increments were plotted against esophageal pressure difference change measured at the ends of the periods of interruption. The slopes of the volume-pressure plots obtained in the region of the normal resting midposition represent "static" pulmonary compliance values if the static state may be presumed to have occurred during the two-second interruption. In Figure 5 the values obtained in this manner in the two patients are plotted as squares on the ordinates. The "X" marks indicate the mean of values measured during quiet respiration and the dots represent compliance values during voluntary hyperventilation. The "static" compliance is about three times greater than during quiet breathing and some twelve times greater than during hyperventilation.

Although "static" compliance measurements were made on only two of the patients, other evidence obtained in six patients suggests that, under conditions approaching the static stage, pulmonary compliance was increased from quiet breathing levels. During vital capacity determinations the patients were instructed to maintain the maximal inspiratory position with an open glottis for a period of several seconds before expiring maximally. Flow was near zero in these instances and esophageal pressure approached "static" conditions. In only one patient was the intra-esophageal pressure as low as -20 cm. of water. In the remaining patients the pressures ranged from -7 to -11 cm. of water. In five normal individuals the pressure ranged from -25 to -33 cm. of water. At the instant of maximal inspiration the patients had somewhat smaller gas volumes within their lungs than the normals; total capacities in these six patients ranged from 5.2 to 7.9 liters with a mean of 6.6 while in the five normals they ranged from 7.0 to 9.0 with a mean of 8.3. Taking the ratio of the total lung capacity to the intra-esophageal pressure at the maximal inspiratory level as a rough

measure of the over-all pulmonary compliance, the patients with one exception had greater values than the normals. (Patient range: 0.3 to 1.1 liters per cm. of water with a mean of 0.5. Normal range 0.26 to 0.36 with a mean of 0.3).

DISCUSSION

The flow-resistive behavior of emphysematous lungs

Quantitative evidence of increased expiratory flow resistance in emphysema was first obtained in one patient by Neergaard and Wirz (5). Dayman (6), and more recently Fry, Ebert, Stead, and Brown (7) have confirmed this in all of their patients. The same observation has been made in a patient with bronchial asthma (4) and in normal individuals under three specific circumstances: 1) During maximally rapid expirations (1, 7); 2) during quiet breathing accomplished voluntarily well down in the expiratory reserve range (1, 7); and 3) during quiet breathing at a normal midposition following the inhalation of a histamine aerosol, which produced a two- to three-fold increase in respiratory flow resistance (4).

All of these observations are consistent with the "check-valve" mechanism explored in detail by Dayman. Since this mechanism may play a role in the elastic behavior of emphysematous lungs it is discussed here.

During the respiratory cycle the intrathoracic airways are subjected to alterations in pressure difference across their walls. Because the pressure on the outside of the airways more closely approximates intrapleural pressure than the pressure within the airways—which is certainly true for the extrapulmonary portions, the trachea and main bronchi—the pressure difference tends to keep the respiratory tract expanded during quiet respiration. During rapid forced expiration, however, this pressure difference is reversed, and the respiratory tract caliber reduced, thereby increasing pulmonary flow resistance. Furthermore, this sequence of events would be "self-augmenting" since increased velocity of flow would further increase the unfavorable transmural pressure difference. Conversely, during inspiration the events should be "self-limiting." Here the transmural pressure difference is reversed, tending to increase the cross-section which would lead to reduction in air-flow resistance and air-stream kinetic energy and thus reduce the transmural pressure difference. This conclusion is consistent with the observation that no equivalent reductions in pulmonary frictional resistance are observed during rapid inspiration.

Direct observations of the respiratory tract by bronchoscopy and bronchography support this hypothesis. Di Rienzo (8) has demonstrated with bronchograms tracheobronchial collapse during the rapid expiratory phase of cough efforts. Furthermore, invagination of the membranous portion of the trachea and bronchi during rapid expiratory efforts is commonly seen during bronchoscopy.

A number of possible structural changes could result in abnormal compression of the respiratory tract during expiration. Reduction in cross-section of the smaller branches of the respiratory tree could have such a result. The resulting increase in air-flow resistance would be associated with greater transmural pressure fluctuations in the intrathoracic portions of the airway "mouthward" from the points of constriction. Increased tissue frictional resistance could give a similar result.

In addition to mechanisms producing increased transmural pressure fluctuations, two additional possible predisposing factors should be mentioned. The structural integrity of the airway walls *per se*, could play a role to the extent that weakening of the walls would reduce the pressure necessary to cause collapse. Furthermore, as Fry, Ebert, Stead, and Brown have demonstrated (7), the elasticity of the lung plays a protective role in preventing collapse of the airways during expiration.

Of these factors only the last is clearly evident in emphysema, but any or all may play a role. Finally, although expiratory increases in resistance may be more marked in emphysema than in other conditions, they are not limited to emphysema.

The elastic behavior of emphysematous lungs

Christie (9) first noted that the "distensibility of the lungs" in emphysema appeared to be greater when measured from tracings of volume and intrapleural pressure during vital capacity maneuvers than from similar tracings during quiet breath-

ing. Commenting on these findings, McIlroy and Christie (10) said: "The maximum inspirations were made slowly at a rate of approximately five respirations per minute as compared with the 15 to 20 per minute of the patient's normal breathing, and, in fact, these results show that during life the distensibility of the lungs in emphysema depends largely on the respiratory rate." Although "distensibility" in this instance undoubtedly included flow-resistance along with elastic behavior, it is probable that they were describing the same phenomenon here reported.

In attempting to explain the apparent decrease in compliance of emphysematous lungs during increased breathing rates, the influence of the distribution of elastic and flow-resistive properties within the lung on their over-all mechanical behavior has been considered.

The results of an animal experiment are helpful in introducing these considerations. The compliance of a dog's lung was measured at different breathing frequencies produced by a Starling pump connected to the tracheal cannula. Since the chest had been opened, the transpulmonary pressure could be measured by recording the tracheal pressure with reference to the atmosphere. Over a range of frequency from 5 to 60 cycles per minute the compliance remained constant. A catheter with a small balloon around the tip was then passed into the left main bronchus and inflated so as to block completely ventilation of the left lung. The compliance of the right lung was then measured. It was slightly more than one-half as compliant as the two lungs together, as would be expected from the relative size of the two lungs. Again the compliance did not change with frequency.

Next the balloon was slightly deflated permitting some ventilation of the left lung, but the resistance to flow was greater than normal. In this instance when volume changes were produced slowly the two lungs had nearly equal tidal excursions. The compliance was the same as that measured for the two lungs before the catheter was passed. As the rate was increased it was observed that the partially obstructed lung participated less in the total volume change and its movement appeared to lag behind that of the right lung. The compliance measured in this instance had decreased. With further increase in rate, compliance fell to levels nearly the same as recorded for the right lung alone. At these rapid rates the volume change of the left lung was barely noticeable; almost all of the flow was into and out of the right lung.

This experiment illustrates in gross fashion the influence of an abnormal distribution of resistance on the mechanical behavior of lungs. At low frequencies, when the rates of flow were low, the resistance discrepancy had little effect on the distribution of flow to the two lungs. In this instance the compliance of the lungs determined the distribution of volume change, and the over-all system compliance was the sum of the individual lung compliances. As the cycling frequency of the pump was increased the differences in flow resistance to the two lungs had a progressively greater effect on the distribution of flow to the two lungs. Functionally, the apparent reduction in compliance reflected this change.

The concepts illustrated by this experiment can be extended to points further along the respiratory tree. At every point of branching the distribution of flow between the two branches depends on the elastic and flow-resistive properties of the two pathways. When the rates of flow are low this distribution depends chiefly on the elastic properties of the pathways. As the rates of flow increase, the distribution depends increasingly, and at high rates of flow predominantly on the flow resistance of the two pathways.

The only circumstance under which the over-all compliance of the lungs will remain unchanged with changes in breathing rate is one in which the distribution of flow within the lungs remains the same as the breathing frequency changes. It is apparent that flow-resistive and elastic properties would have to be distributed in a special way to permit this. In attempting to understand the nature of such a distribution, techniques developed in the field of electrical engineering are useful. Certain electrical networks are analogous to the lungs' mechanical networks, and the theory that has been developed to account for their behavior can be applied to the lungs. It is not within the scope of this report to present such an analysis.[5] The conclusions which may be reached can be expressed simply:

[5] Details of this analysis will appear in Reference 4.

Considering any pathway in the lung, the product of the flow resistance and compliance of the pathway has the unit of time:

$$\text{Compliance} \times \text{Resistance} = \text{Time}$$

$$\frac{\text{Volume}}{\text{Pressure}} \times \frac{\text{Pressure}}{\text{Volume/Time}} = \text{Time}$$

This product is called the time-constant of the pathway. It can be shown theoretically and demonstrated experimentally, that if the *time-constants* of all the separate pathways are equal the distribution of flow within a system such as the lungs is independent of frequency. Only under this circumstance is the measured over-all compliance also independent of frequency. Any time-constant inequality, whether the result of changes in local elastic properties, flow resistive properties, or a combination of both, will result in changes in the distribution of flow with changes in frequency, and reduction in the over-all compliance as frequency is increased.

Certain corollaries of this analysis may be mentioned. The finding of unchanged compliance with changes in breathing rates in normal subjects suggests that the distribution of ventilation in normal lungs also remains the same over a wide range of frequencies. By this, equality of ventilation to all parts of the lung is *not* implied. It merely means that the distribution, such as it is, does not change. In contrast, the marked reduction in compliance with increasing frequency observed in emphysema implies that even during quiet breathing a considerable part of the uneven ventilation, known to exist in emphysema, must relate to dynamic factors. In all probability these abnormalities are emphasized with increasing ventilation during exercise.

It is not possible to say whether local changes in elastic or resistive properties, or both (including the check valve mechanism) are responsible for the changes in compliance with changing frequency in emphysema. It is of interest to speculate about the possible influence of this phenomenon on the pathogenesis of the disease. If the distribution of increases in air-flow resistance is non-uniform within the lungs, those parts of the lungs with the lowest resistance would become abnormally stressed, since they would undergo greater than normal volume change. The high resistance parts of the lungs would tend to be protected. Large and relatively rapid changes in volume, as during the inspiratory phase of cough, would tend to accentuate this differential stress within the lungs. Thus, it is possible that this phenomenon may play a role in the process of parenchymal destruction and lead from asthma and chronic bronchitis to emphysema.

The differential diagnosis between pulmonary emphysema and bronchial asthma is important since the *former* implies irreversible structural damage while the *latter* does not. From the standpoint of pulmonary mechanics the two conditions have much in common. In both conditions increases in pulmonary flow resistance are greatest during expiration and pulmonary compliance falls with increased rates of breathing. Theoretically certain mechanical differences should be measurable. The static compliance of the lungs of the patient with emphysema, as indicated roughly by the extent to which he can lower his intra-esophageal pressure during a slow maximally deep inspiration, should be higher than normal. (The presence of cor pulmonale in emphysema might negate this distinction.) Furthermore, the asthmatic patient studied on his "best day" or following the administration of bronchodilators might be expected to show little change in compliance with frequency and near-normal pulmonary flow resistance, while the patient with emphysema under similar circumstances would still show marked mechanical abnormality.

SUMMARY

Measurements of the mechanical properties of the lungs in ten patients with advanced pulmonary emphysema were compared with similar measurements in ten normal subjects.

Pulmonary flow resistance was found to be increased in emphysema, more so during expiration than during inspiration. Pulmonary flow resistance during expiration was further increased by increased effort.

In the patients, pulmonary compliance was near normal levels during quiet breathing, but fell markedly as the breathing frequency was increased.

Possible local mechanisms accounting for these changes are discussed and the implications of the distribution of mechanical properties within the

lungs on their over-all mechanical behavior considered.

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THE EFFECT OF ORAL PROTEIN AND GLUCOSE FEEDING ON SPLANCHNIC BLOOD FLOW AND OXYGEN UTILIZATION IN NORMAL AND CIRRHOTIC SUBJECTS [1, 2]

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Since the dietary management of patients with cirrhosis is of great importance it was felt that assessment of the vascular and metabolic responses of the splanchnic area to protein and glucose feeding would shed some light on reactions of the splanchnic vasculature and of the liver parenchyma which might be therapeutically desirable, or the reverse.

METHODS

The subjects for this investigation were patients from the Medical Wards of the University Service, Kings County Hospital. A total of 40 patients were studied. Fourteen patients who had recovered from recent acute infections or other minor illnesses and were considered to have normal liver function by the usual clinical and laboratory criteria, served as a control group. There were 26 subjects in the cirrhotic group. Twenty-one of these cirrhotic patients had a history of prolonged excessive alcoholic intake and poor nutrition; 5 had a history of acute hepatitis years previous to the onset of their present illness. The diagnosis of cirrhosis was supported by laboratory studies in all cases, and in 15 subjects by liver biopsy. Ten of the cirrhotic patients had ascites and paracenteses were performed 24 hours before study so that they were as "dry" as possible during the study.

The estimated splanchnic blood flow (ESBF) and splanchnic oxygen consumption (SOC) were determined in all subjects in the morning after a fast of at least 12 hours, and for variable periods up to 90 minutes following the ingestion of either protein or glucose. The splanchnic blood flow was determined by the bromsulfalein (BSP) method (3). An infusion of BSP (in 0.85 per cent saline) was administered intravenously at a constant rate maintained in a few instances by a tunnel clamp and in the remaining cases by a calibrated Bowman pump. The rate of infusion was adjusted so that the peripheral BSP concentration was maintained at a constant level throughout each study below 2.0 mgm. per cent following a priming dose of 150 mgm. A specially designed double lumen venous catheter in which the lumina are 3 inches apart was introduced into the right hepatic vein under fluoroscopic control, advanced as far as possible, and was kept in that position throughout each study. All blood sampling was done from the proximal opening, which was positioned in a branch of the hepatic vein. Samples of blood for arterial oxygen content and BSP concentration were obtained through an indwelling arterial needle in either the femoral or brachial artery. Thirty to 60 minutes after the establishment of proper BSP levels 3 or 4 simultaneous samples of arterial and hepatic venous blood were obtained at 5 to 10-minute intervals for colorimetric determination of plasma bromsulfalein concentration. Samples of arterial and hepatic venous blood were also obtained without exposure to air for the determination of oxygen differences. Care was taken to assure a free flow of blood from the catheter so as to prevent inadvertent dilution and thus invalid determinations.

The estimated splanchnic plasma flow was calculated from extrapolated data midway between two successive determinations of arterial and hepatic vein BSP plasma concentrations. The splanchnic oxygen consumption (SOC) was calculated only in those instances when arterial and hepatic vein oxygen samples were obtained within five minutes of this extrapolated midpoint.

In those instances where the peripheral BSP concentration was changing more than 0.003 mgm. per cent per minute (ΔP) the data were considered invalid and discarded. In those instances where the arterial minus hepatic vein (A-HV) BSP concentration divided by arterial BSP concentration, E_{BSP} per cent, that is the hepatic extraction of bromsulfalein, was less than 10 per cent during control periods, blood flow was not estimated. In both normal and cirrhotic subjects fed protein the BSP concentration in the hepatic vein rose with no detectable change in peripheral BSP levels indicating a decrease in E_{BSP} per cent. In a few instances there was a concomitant slight rise in peripheral BSP levels with the fall in E_{BSP} per cent indicating a decrease in over-all BSP clearance. Estimated splanchnic blood flow was determined from values for estimated plasma flow and arterial hematocrits which were measured in Wintrobe

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[2] Portions of this work were presented at the Eastern Section Meeting, American Federation for Clinical Research, Boston, January 4, 1954 (1) and have appeared in abstract (2).

tubes centrifuged at 3,000 rpm for 30 minutes. It was assumed that the peripheral arterial and splanchnic hematocrits maintained a constant relationship to each other throughout each study. Data for both ESBF and SOC have been converted to rates per square meter of body surface. Per cent changes represent the increases or decreases from average control values. All data were discarded if the splanchnic blood flow in any one of three successive control periods varied more than 10 per cent from the mean ESBF of these periods. Recorded post feeding data are single determinations.

In the cirrhotic group fed protein there were seven subjects with hyperbilirubinemia in whom ESBF was not measured. Blood was drawn from the collateral abdominal veins of two cirrhotic patients for the determination of amino acid nitrogen content.

Following three control periods, the subjects in whom the effects of protein feeding were to be studied, ingested 250 grams of chopped lean beef which contained 60 grams of protein. The meat was broiled, homogenized in a Waring blendor with water and salt, and then reheated. The consistency of the meal was approximately that of a thick soup and could easily be taken through a glass straw by the patient while he remained in the supine position. In the glucose feeding studies the patients ingested 100 grams of glucose to which lemon juice had been added. Protein and glucose feedings were of approximately equal volume (500 cc.), but were not isocaloric.

After either of these two feedings the sampling of hepatic venous and arterial blood permitted determinations of bromsulfalein and oxygen, and in certain instances amino acid nitrogen and glucose content. Since carefully selected beef was used for protein feeding, alimentary lipemia did not interfere with the colorimetric determinations of BSP. Sampling was started as early as five minutes after the feeding in some instances, and as late as 30 minutes afterwards in others. Specimens were obtained at approximately 10 to 15-minute intervals up to 90 minutes after the ingestion of the food.

Arteriovenous oxygen differences were determined once during the control periods, again during the first 50 minutes after feeding, and at least once more before the study was terminated. The oxygen concentrations were determined by the manometric method of Van Slyke and Neill (4); glucose concentrations from zinc sulfate filtrates by a modification of the Somogyi method (5); amino acid nitrogen from tungstate filtrates by a modification of the Folin-Wu method (6).

TABLE I

The effect of oral protein feeding in normal subjects

Subject		Fasting control	Time (minutes) after feeding							
			20	30	40	50	60	70	80	90
N. K.	ESBF	1090		1575(45)*	1510(39)	1370(26)	1190(9)			
	SOC	37		46(24)			48(30)			
(M)	A-HV O_2	3.4		2.9			4.0			
V. G.	ESBF	920			980(7)	1240(35)	1220(33)	1215(32)	1160(26)	
	SOC	33			42(28)				57(73)	
(M)	A-HV O_2	3.6			4.3				4.9	
J. B.	ESBF	1340				1780(33)		1760(31)	1755(31)	1485(11)
	SOC	59				70(19)		92(56)		77(31)
(M)	A-HV O_2	4.4				3.9		5.2		5.2
W. O'C.	ESBF	1205			1430(19)	1365(13)	1205(0)	1105(−8)		
	SOC	45				74(64)		69(53)		
(M)	A-HV O_2	3.7				5.4		6.2		
C. S.	ESBF	970				1225(26)		1190(23)	1135(17)	1070(10)
	SOC	28				45(61)		36(29)		
(F)	A-HV O_2	2.9				4.3		3.0		
S. P.	ESBF	1130	1800(59)	1760(56)	1670(48)	1730(53)		1690(50)	1755(55)	1705(51)
	SOC	70			95(36)				109(56)	
(M)	A-HV O_2	6.1			5.7				5.4	
A. W.	ESBF	1480		2000(35)		2130(44)	2080(41)		1820(23)	
	SOC	49		82(67)					64(31)	
(M)	A-HV O_2	3.3		4.1					3.5	
Average	ESBF	1160	Average† of 20		1570(35)		Average† of 60		1430(23)	
of	SOC	46	through 50-minute		65(41)		through 90-minute		69(50)	
control	A-HV O_2	3.9	period		4.4		period		4.7	

* Data in parentheses represent percentage increases or decreases (−) from control.

† The average ESBF recorded was derived from average data of each individual during the two main time periods; *e.g.*, the ESBF for subject N. K. during 10 through 50-minute period was 1485, and 1190 during 60 through 90-minute period.

TABLE II

The effect of oral protein feeding in cirrhotic subjects

A. Non-icteric

Subject		Fasting control	Time (minutes) after feeding 10	20	30	40	50	60	70	80	90
J. W.	ESBF	380				445(17)	495(30)	405(7)	360(−5)		435(12)
	SOC	21				21(0)					30(43)
(M)	A-HVO$_2$	5.4				4.7					7.0
J. T.	ESBF	1075					1390(29)	1160(8)	940(−13)		
	SOC	36					54(50)		30(−17)		
(M)	A-HVO$_2$	3.3					3.9		3.2		
A. McC.	ESBF	725				840(16)	910(26)	980(35)			
	SOC	51				40(−22)		63(24)			
(M)	A-HVO$_2$	7.1				4.8		6.4			
C. M.	ESBF	1025	1065(4)		1130(10)			1090(6)	1130(10)		1220(19)
	SOC	70			67(−4)				70(0)		89(27)
(M)	A-HVO$_2$	6.9			5.9				6.2		7.3
W. R.	ESBF	780		840(8)	950(22)		935(20)		945(21)		800(3)
	SOC	42		48(14)					53(26)		
(M)	A-HVO$_2$	5.2		5.7					5.6		
T. D.	ESBF	570			985(73)	865(52)	750(32)	785(38)		930(63)	930(63)
	SOC	16				37(131)					28(75)
(M)	A-HVO$_2$	2.8				4.3					3.0
J. R.	ESBF	1400		1510(8)	1710(22)	1305(−7)		1275(−9)			
	SOC	45			55(22)			42(−7)			
(M)	A-HVO$_2$	3.2			3.2			3.3			
P. A.	ESBF	410	415(1)		445(8)						
	SOC	24			29(21)						
(M)	A-HVO$_2$	5.8			6.2						
E. A.	ESBF	905			1450(60)			1260(39)			
	SOC	44			64(45)			52(14)			
(M)	A-HVO$_2$	4.9			4.5			4.1			
N. J. (M)	A-HVO$_2$	6.6	6.3								6.2
E. I. (M)	A-HVO$_2$	6.2			6.8				6.1		
I. T. (M)	A-HVO$_2$	3.8		5.5				4.6			
L. S. (M)	A-HVO$_2$	2.6			3.3						
Average of control	ESBF	810	Average of 10 through 50-minute period			1000(23)		Average of 60 through 90-minute period			985(21)
	SOC	39				46(18)					51(31)
	A-HVO$_2$	4.9				5.0					5.3

RESULTS

Since all control data represent similar fasting conditions for both types of experiment (protein and glucose feeding) the results are calculated as grouped data in normal and cirrhotic subjects. In each table, however, recorded averages were derived from data in the particular table.

Control periods

The mean control ESBF for 10 normal subjects (Table I, 7 subjects and Table IIIA, the first 3 subjects) was 1130 cc. per min. per m^2 ± 340 cc. The mean SOC for these 10 normal subjects was 43.5 cc. per min. per m^2 ± 7.5 cc. The mean A–HV oxygen difference for 14 normal subjects

TABLE II—*Continued*

B. Icteric

Subject		Fasting control	Time (minutes) after feeding								
			10	20	30	40	50	60	70	80	90
W. L. (M)	A-HVO_2	3.3				3.1			3.7		
J. V. (M)	A-HVO_2	3.3				4.5			5.3		
D. D. (M)	A-HVO_2	1.5				1.2					2.2
W. B. (M)	A-HVO_2	2.3								4.3	
A. A. (M)	A-HVO_2	1.9			4.0			4.0			
P. H. (M)	A-HVO_2	2.4		2.8				2.8			
H. J. (M)	A-HVO_2	2.8			3.7						
Average	A-HVO_2 = 2.5		Average of 10 through 50-minute period = 3.2						Average of 60 through 90-minute period = 3.5		

(10 subjects cited above plus the last 4 subjects, Table IIIA) was 4.0 vol. per cent ± 0.8 cc.

The mean control ESBF for 11 non-icteric cirrhotic subjects (Table IIA, the first 9 subjects and Table IIIB, the first 2 subjects) was 730 cc. per min. per m² ± 340 cc. The mean SOC for these 11 subjects was 40.5 cc. per min. per m² ± 16 cc. The mean A–HV oxygen difference for 19 non-icteric cirrhotic subjects (11 subjects cited above plus the last 4 subjects, Table IIA and the last 4 subjects, Table IIIB) was 4.8 vol. per cent ± 1.4 cc. In the 7 cirrhotic subjects with clinical icterus (Table IIB), the mean A–HV oxygen difference was 2.5 vol. per cent ± 0.7 cc.

Post feeding results

A. Protein. Tables I, IIA, and IIB list the effects of protein feeding in normal and cirrhotic subjects.

In the group of seven normal subjects (Table I) the average ESBF increased from a fasting value of 1160 cc. per min. per m² to 1570 cc. per min. per m², 35 per cent above the control in the first hour after the protein feed. During the next thirty minutes there was some return towards control levels, the ESBF averaging 1430 cc. per min. per m², 23 per cent above control. There was also an increase in the average A–HV oxygen difference for the normal subjects from a control value of 3.9 vol. per cent to 4.4 vol. per cent during the first hour after feeding and a further increase to 4.7 vol. per cent in the next 30 minutes. The average fasting SOC of 46 cc. per min. per m² rose to 65 cc. per min. per m², a 41 per cent increase, during the first post feeding hour and reached 69 cc. per min. per m², to a 50 per cent increase during the next thirty minutes. Although there was a slight decrease in the average ESBF during the last thirty minutes of observation, as compared with the ESBF of the first hour, the increased A–HV oxygen difference at the time was greater so that the SOC increased.

In 9 non-icteric cirrhotic subjects (Table IIA) the ESBF rose from an average level of 810 cc. per min. per m² in the fasting state to 1000 cc. per min. per m², 23 per cent above control, during the first hour post feeding, and fell slightly to 985 cc. per min. per m², still 21 per cent above control during the final 30 minutes of observation. A–HV oxygen differences in the 13 non-icteric cirrhotic subjects (Table IIA) changed insignificantly after feeding from an average fasting level of 4.9 vol. per cent to 5.0 vol. per cent during the first hour and to 5.3 vol. per cent in the next 30 minutes. From an average fasting SOC of 39 cc. per min. per m² there was an increase to 46 cc. per min. per

TABLE III

The effect of oral glucose feedings

A. Normal subjects

Subject		Fasting control	Time (minutes) after feeding 10	20	30	40	50	60	70	80	90
L. C. (M)	ESBF	1250†		1180(−6)	1150(−8)	1045(−16)		1170(−6)			
	SOC	37.5			31(−17)			33(−12)			
	A-HVO_2	3.0			2.6			2.8			
	PER.GL.*	71		95	108	118		107			
E. D. (M)	ESBF	1050		945(−10)	930(−11)		960(−8)	980(−6)		990(−6)	1010(−4)
	SOC	47			35(−26)						40(−15)
	A-HVO_2	4.5			3.8						4.0
	PER.GL.	94		123	112		115	110		100	90
F. McG. (F)	ESBF	890	850(−4)	840(−6)		880(−1)	845(−5)	860(−3)			
	SOC	30		31(3)				27.5(−8)			
	A-HVO_2	3.4		3.7				3.2			
	PER.GL.	86	98			128	120	115			
J. L. (M)	A-HVO_2	4.5		3.0					3.7		
W. M. (M)	A-HVO_2	4.2				2.9			3.1		
E. P. (M)	A-HVO_2	4.2				3.8					
A. M. (M)	A-HVO_2	5.5				4.8			4.8		
Average of control	ESBF	1065	Average‡ of			975(−8)		Average of			1010(−5)
	SOC	38	10 through 50-			32(−16)		60 through 90-			33.5(−12)
	A-HVO_2	4.2	minute period			3.5		minute period			3.6

B. Cirrhotics

Subject		Fasting control	Time (minutes) after feeding 10	20	30	40	50	60	70	80	90
N. P. (M)	ESBF	590			540(−8)		590(0)				
	SOC	38			32(−16)						
	A-HVO_2	6.5			5.9						
	PER.GL.	80			124		120				
P. M. (M)	ESBF	1175		700(−40)	825(−30)			930(−21)			
	SOC	59		24.5(−58)				48(−19)			
	A-HVO_2	5.0		3.5				5.2			
	PER.GL.	74		112				80			
N. L. (M)	A-HVO_2	4.0				4.4		3.4			
A. K. (M)	A-HVO_2	3.6			3.7			2.8			
S. K. (M)	A-HVO_2	4.0			2.9			2.9			
C. G. (M)	A-HVO_2	4.3		3.7						2.9	
Average of control	ESBF	880	Average of			—		Average of			—
	SOC	48	10 through 50-			—		60 through 90-			—
	A-HVO_2	4.6	minute period			4.0		minute period			3.4

* Peripheral (arterial) blood glucose level, mgm. per cent.

† Recorded control ESBF in each subject is the average of three successive determinations.

‡ Recorded average ESBF in the 10 through 50-minute and in the 60 through 90-minute periods was obtained by using the average ESBF of each subject during these two intervals, *e.g.*, the ESBF in patient L. C. in the 10 through 50-minute period averaged 1125 cc.

m^2 or 18 per cent above control during the first hour after the meal, and then a very slight further increase to 51 cc. or 31 per cent above the control during the last 30 minutes of observation.

In the seven icteric cirrhotic subjects (Table IIB) there was a progressive increase in A–HV oxygen difference from a low average fasting level of 2.5 vol. per cent to 3.2 vol. per cent during the first hour post feeding, and to 3.5 vol. per cent during the next 30 minutes of observation.

In subjects I. T. and L. S. (Table IIA) with large abdominal collateral veins, amino acid nitrogen concentration rose in these vessels from fasting levels of 7.0 and 5.8 mgm. per cent to 15.4 and 14.8 mgm. per cent, respectively, twenty minutes after a protein feed. At one hour the levels were 16.0 and 17.0 mgm. per cent, respectively.

TABLE IV

Blood amino acid nitrogen levels in peripheral artery and hepatic vein before and one hour after protein feeding

A. Cirrhotics

Subject	Time	Periph. Art.	Hep. Vein
P. A.	Cont.	6.0	4.8
	1 hour	8.0	7.4
E. A.	Cont.	6.3	9.5
	1 hour	12.1	12.9
N. J.	Cont.	6.9	7.0
	1 hour	10.7	12.0
P. H.	Cont.	7.7	6.1
	1 hour	9.8	9.1
W. L.	Cont.	5.8	5.2
	1 hour	9.9	12.4
A. A.	Cont.	6.6	6.0
	1 hour	11.1	10.9
J. R.	Cont.	5.6	5.6
	1 hour	6.5	5.9
E. I.	Cont.	6.9	5.9
	1 hour	12.4	8.2
Average	Cont.	6.5	6.2
	1 hour	10.1	8.6

B. Normals

A. W.	Cont.	6.3	6.1
	1 hour	6.5	6.0
J. B.	Cont.	7.0	6.9
	1 hour	7.2	7.0
Average	Cont.	6.7	6.5
	1 hour	6.8	6.5

In eight cirrhotic subjects (Table IV) the peripheral amino acid nitrogen concentrations rose from an average fasting level of 6.5 mgm. per cent to 10.1 mgm. per cent one hour after protein ingestion while the corresponding hepatic vein concentrations increased from 6.2 mgm. per cent to 8.6 mgm. per cent in the same interval. In two of the eight subjects amino acid nitrogen levels in the hepatic vein were significantly higher than their corresponding peripheral levels one hour after the protein feed. In two normal subjects there was no significant change in amino acid nitrogen levels in either arterial or hepatic vein blood one hour after the feeding.

B. Glucose. In three normal subjects (Table IIIA) there was a slight fall in ESBF during the first hour after the glucose feeding from an average fasting level of 1065 cc. per min. per m^2 to 975 cc. per min. per m^2. This represented an 8 per cent fall in flow. During the next thirty minutes of observation there was a tendency toward a return to the control levels, the average ESBF during this period being 1010 cc. per min. per m^2. A–HV oxygen differences showed a somewhat more striking effect, falling from an average of 4.2 vol. per cent to 3.5 vol. per cent during the first hour of observation, and remaining quite constant at 3.6 vol. per cent during the next 30 minutes. The splanchnic consumption of oxygen for these three normal subjects fell slightly after glucose feeding from an average fasting level of 38 cc. per min. per m^2 to 32 cc. per min. per m^2 during the first hour of observation and changed but little during the next 30 minutes averaging 33.5 cc. per min. per m^2. Glucose tolerance as measured by peripheral blood levels was at all times within the normal range. The alterations in hepatic blood flow and oxygen consumption were sustained beyond the time at which peripheral blood glucose concentrations (see Table III) had returned to fasting levels.

The effect of glucose feeding upon ESBF and SOC was studied in two cirrhotic subjects. In subject N. P. (Table IIIB) there was a slight initial fall in the ESBF 30 minutes after the glucose feeding from a fasting level of 590 cc. per min. per m^2 to 540 cc. per min. per m^2 with return to the control level 50 minutes after ingestion of glucose. There was a slight fall in oxygen consumption 30 minutes after the ingestion of glucose from a

fasting level of 38 cc. per min. per m^2 to 32 cc. per min. per m^2. In subject P. M. (Table IIIB) there was a more striking fall in blood flow in the first 30 minutes after the ingestion of glucose to 765 cc. per min. per m^2 from a control level of 1175 cc. per min. per m^2. One hour after the insplanchnic oxygen consumption was 24.5 cc. per min. per m^2, 21 per cent below the control level. Twenty minutes after the ingestion of glucose the splanchnic oxygen consumption was 24.5 cc. per min. per m^2, having decreased to less than half of control level which was 59 cc. per min. per m^2. The fall in SOC was noted when the peripheral glucose level was elevated, and 60 minutes after the administration of glucose, when the peripheral blood glucose had returned to the fasting level, the SOC was 48 cc. per min. per m^2, 19 per cent below the control value.

In the six cirrhotic subjects (Table IIIB) fed glucose A–HV oxygen differences fell from an average control level of 4.6 vol. per cent to 4.0 vol. per cent during the first hour of observation and subsequently to 3.4 vol. per cent during the next 30 minutes.

DISCUSSION

From the present study of the short term effects of protein and glucose feeding in normal and cirrhotic subjects certain conclusions seem warranted. First, after the ingestion of protein there is an increase in estimated splanchnic blood flow, arterio-hepatic vein oxygen difference, and splanchnic consumption of oxygen in both normal and cirrhotic subjects. However, although the changes are qualitatively similar the increases occur somewhat sooner and are of greater magnitude in the normal as compared with the cirrhotic patient. Second, glucose ingestion results in minimal changes in all parameters measured in both normal and cirrhotic subjects. Third, the liver of the fasting, icteric, cirrhotic subject extracts much less oxygen per unit of blood than does the liver of the fasting normal, or of the non-icteric, cirrhotic subject. Following the ingestion of protein by the cirrhotic subject with icterus, the A–HV oxygen difference increases more in absolute values and in percentage changes than it does in the normal, or in the non-icteric cirrhotic subject.

In the fasting state there is little difference in splanchnic oxygen consumption between the cirrhotic and the normal subject despite the lessened blood flow in the former because of the greater oxygen extraction from each unit of blood. During these acute studies following the ingestion of protein, the A–HV oxygen difference increases significantly in the normal subject whereas in the cirrhotic subject with approximately the same percentage increase in blood flow, extraction of oxygen per unit of blood increases minimally. This failure of the cirrhotic liver to increase oxygen extraction with increased blood flow may be due to hepato-cellular disease, intrahepatic and extrahepatic shunting of blood, or to perfusion of acellular and non-extracting tissue.

The specific dynamic action of protein has been shown in experimental animals to be due to excess metabolism in the splanchnic region (7, 8). This finding has been confirmed in humans by the intravenous administration of amino acids which cause great increases in splanchnic oxygen consumption and little or no change in splanchnic blood flow (9). In the present study the feeding of protein was followed by a significant increase in splanchnic oxygen consumption, similar to that caused by the intravenous administration of amino acids; however, a significant increase was also noted in ESBF. The effect on ESBF of oral amino acids which require no digestion is not known. It would seem unlikely that the increase in ESBF which occurs following the ingestion of protein is related to the increased SOC associated with the metabolism of amino acids. The vascular and metabolic responses of the splanchnic area would appear to depend in part upon the route of administration of a given substance, *e.g.*, the effects of alcohol upon splanchnic blood flow differ with the oral and intravenous routes of administration (10, 11). The metabolic processes associated with deamination and the production of urea probably add to the oxygen requirements of the liver. In as short a period as 20 minutes after the ingestion of protein, estimations of the amino acid nitrogen content of blood taken from collateral venous channels in the two patients referred to previously showed a definite rise. The rapidity of the changes noted in SOC after the ingestion of protein in this study may be partly dependent upon the specific dynamic action of amino acids. An

SDA effect need not necessarily be accompanied by a rise in ESBF (9).

The icteric cirrhotic subjects exhibited very low arterio-hepatic vein oxygen differences in the fasting state and, although these differences increased considerably following the ingestion of protein when measured in percentages or in absolute values, they failed to approach the levels found in either normal or non-icteric cirrhotic subjects. It would appear likely that these observations reflect the severe degree of hepato-cellular dysfunction present in cirrhotic patients with jaundice.

The oral administration of considerable quantities of glucose is not accompanied by significant change in the parameters used in this acute study to demonstrate vascular or metabolic changes in the splanchnic area. Intravenous glucose reduces splanchnic oxygen consumption and blood flow (9). The observed minimal change in splanchnic oxygen consumption may be explained by the conversion of ingested glucose to fat and fatty acids (12) freeing oxygen equivalent to 5 calories per gram of glucose converted. The ingestion of glucose will reduce endogenous protein metabolism in a fasting liver and thus urea production and deamination will diminish (13). It may therefore be possible to achieve a relatively high caloric intake without causing an increase, but perhaps a decrease, in splanchnic oxygen consumption and/or blood flow. Indeed, our short term data suggest that a fall in splanchnic oxygen consumption may follow the ingestion of glucose. The effects of complex polysaccharides which require digestion have not been studied.

A growing body of evidence has cast suspicion on the role of metabolites of nitrogenous substances in the genesis of hepatic coma (14). Ammonia levels of hepatic vein blood are higher in cirrhotic subjects than in normal individuals after the ingestion of a standard dose of ammonium chloride (15). Small increments in the protein intake of apparently stabilized cirrhotic patients may precipitate hepatic coma (16). These phenomena would appear to be related to the shunting of gastrointestinal venous blood directly into the systemic circulation or to the failure of adequate metabolism by a diseased liver. The present study has demonstrated that the amino acid nitrogen levels of hepatic vein and peripheral blood are higher in cirrhotic patients than in normals after a protein feed. This too is a reflection of the combination of hepato-cellular disease and vascular shunting.

The uniform rise in ESBF following protein feeding may be related to changes in portal vein pressure. Data obtained in our laboratory demonstrate (17) that protein feeding is often followed by a rise in hepatic venule wedge pressure in normals and more so in cirrhotic subjects, whereas glucose feeding does not produce this effect. Hepatic wedge pressure is an excellent reflection of portal vein pressure (18). Since further increases in portal vein pressure in patients with hepatic cirrhosis complicated by portal hypertension are undesirable, this consequence of protein ingestion must be considered in the management of such patients.

To decrease the work of a diseased organ is a basic therapeutic concept. One of the current treatments for hepatic cirrhosis includes the administration of large amounts of protein (19). If increased splanchnic oxygen consumption or work is considered to be deleterious, then it may well be that a high protein intake is unnecessary and perhaps injurious in patients with hepatic cirrhosis. Although no one denies the need for protein repletion in many patients with hepatic cirrhosis and though it is often difficult to correlate the results of acute experimentation with the long term management of a chronic disease, it would now seem, from several points of view, that a nutritious diet with quantities of protein of high biologic value just sufficient to maintain these patients in positive nitrogen balance might be more advantageous than regimens which stress high protein feeding. Glucose causes no increase in splanchnic oxygen consumption or work and so may be a very valuable constituent of the diet of patients with this disease.

CONCLUSIONS

1. The effects of oral protein and glucose feedings on estimated splanchnic blood flow, splanchnic utilization of oxygen, arterio-hepatic vein oxygen differences have been studied in normal and cirrhotic subjects. These feedings provoke the following responses:

a) In both normal and cirrhotic subjects, protein feeding induced an increase in splanchnic

blood flow, splanchnic oxygen consumption, and A–HV oxygen differences as compared with their fasting levels. Though the effects are qualitatively the same they are less marked and more delayed in the cirrhotic subject than in the normal individual.

b) In normal and cirrhotic subjects, oral glucose feeding induced little change in splanchnic blood flow, oxygen consumption, and arterio-hepatic vein oxygen differences as compared with their control level.

c) There is a small arterio-hepatic vein oxygen difference in the fasting icteric cirrhotic subject, but an increase of this difference occurs following a protein feeding.

2. Amino acid nitrogen levels of peripheral and hepatic vein blood are higher in cirrhotics than in normals after protein feeding.

3. The clinical implications of these observations are discussed.

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THE ABSORPTION OF FATS STUDIED IN A CHILD WITH CHYLOTHORAX

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The way in which fat is absorbed in healthy people is still largely unknown. This lack of knowledge chiefly concerns the question whether complete hydrolysis of the dietary fat is necessary before it is absorbed, or whether absorption through the intestinal wall is possible without complete hydrolysis. In the past few decades this problem has been studied mainly by Verzár and McDougall (1, 2) and by Frazer (3–12), although in widely different ways.

The quintessence of Verzár's theory is: complete hydrolysis of fat in the intestine and synthesis of the fatty acids to triglycerides in the intestinal cells, *via* an intermediate stage of phospholipids. The main features of Frazer's theory are: hydrolysis of part of the fat into mono- and diglycerides and free fatty acids in the intestine, formation of an emulsion and absorption of tiny droplets of fat. The essential point of difference between these two theories is the degree of hydrolysis of the fat in the intestine. This problem has been approached in various ways.

The degree of hydrolysis was determined directly by examination of the intestinal contents after administration of fat by Mattson, Benedict, Martin, and Beck (13), Desnuelle and Constantin (14) and Kuhrt and other workers (15). Other attempts to determine the degree of hydrolysis using an indirect method of calculation, by comparison of chyle fat with dietary fat labelled with isotopes, were made by Borgström (16), Reiser, Bryson, Carr, and Kuiken (17, 18) and Bernhard, Wagner, and Ritzel (19). All these investigations led to the conclusion that there is a considerable hydrolysis of fat in the intestinal lumen. According to Frazer (10) about 40 per cent of the dietary fat is hydrolyzed partially, but Borgström and Reiser consider it highly probable that the hydrolysis, in any case into mono- and diglycerides, is practically complete. The latter authors assume that 25 to 45 per cent of the dietary fat is completely broken down into free fatty acids and glycerol; the remaining 55 to 75 per cent is, according to them completely, and according to Bernhard partially, hydrolyzed into monoglycerides.

There is, however, quite another aspect to this problem. Frazer (6–9), Bernhard (20), Borgström (21, 22), Bloom, Chaikoff, and Reinhardt (23) and Favarger (24) have shown that not all fatty acids are absorbed in the same manner.

According to Frazer the fatty acids of low molecular weight are absorbed in a different manner from those of high molecular weight. Frazer also deals separately with the unsaturated fatty acids, such as Δ^9-octadecanoic acid. As the dietary fats may be of widely varying composition, the determination of the individual absorption of *all* fatty acids, of which the dietary fat concerned is composed, is of great advantage, in contrast to the determination of the total absorption of fat or of the absorption of one particular labelled fatty acid.

The method of Boldingh (25, 26) enables such an analysis. A case of chylothorax in a child offered us a welcome opportunity to use this method in order to study the absorption of the separate fatty acids (27).

CLINICAL FEATURES

Chylothorax is a rare anomaly, arising when the pleural cavity of one or both lungs fills with chyle. The anomaly is caused by a lesion of a chyle vessel. The patient who gave us the opportunity of conducting this investigation was the third child of healthy parents; he was born on July 13, 1950. The other children were healthy. The mother's pregnancy was uneventful; parturition was normal. Directly following birth it was noticed that the patient had a swollen abdomen and a bilateral testicular hydrocele. When the boy was two months old, operation on a bilateral inguinal hernia (which had developed gradually) revealed that the swollen abdomen was due to chyloascites. Seven months later, a right-sided chylothorax was found in addition.

The signs and symptoms of chylothorax and chyloascites alternated in predominance, without any operation being necessary, up to the age of nearly two years. Then he began to have attacks of dyspnea until he was admitted to the hospital, because of the severity of the dyspnea, at the age of 22 months.

On examination no other abnormalities were found apart from a barrel-shaped thorax and much fluid in the right pleural cavity. There was no fluid in the abdominal cavity. Feces, urine and blood were normal on morphological and chemical examination. Liver function and fat balance were also normal (the latter had been disturbed only for a short period during and after an infection). Although the patient's nutritional condition was not optimal, his growth during his affection and after recovery from it was always normal. On the strength of these data the patient was regarded as a healthy child with a purely anatomical abnormality. It may therefore be assumed that the chyle of this patient was also normal.

The right pleural cavity was drained weekly by aspiration during his eight months' stay in the Pediatrics Department. The secretion of chyle into the pleural cavity diminished during the last few weeks of this period and had ceased completely at the end of the eight months. So far relapses have remained absent and, at the time of writing, the child is in good condition.

The patient's chyle came up to the requirements of Baldridge and Lewis (28): 1) its color was that of milk; 2) a cream-like upper layer separated from the fluid at rest; 3) it was an emulsion of fine fat droplets; 4) its reaction was alkaline; 5) the fluid was sterile and not liable to decomposition; 6) the fat content was usually high, varying between 0.4 and 4.0 per cent; 7) the protein content was usually more than 3.0 per cent; 8) the fluid contained many lymphocytes. It appeared moreover that: the fat content of the chyle was dependent on the fat content of the diet; and, the fatty acid composition of the chyle fat was dependent on the fatty acid composition of the dietary fat. These relationships are characteristic of chyle and constitute proof that the fluid aspirated from the thoracic cavity was actually chyle. Results of laboratory examinations are shown in Table I.

TABLE I

Some characteristics of the patient's chyle

	Number of determinations	Range	Average
Cells (mainly mononuclears)	18	(900–6000)	3000/mm³.
Protein (Kjeldahl)	6	(3.37–4.57)	4.00 gm. %
Albumin	6	(1.79–2.54)	2.25 gm. %
($\alpha+\beta$)-Globulin	6	(0.14–0.60)	0.50 gm. %
γ-Globulin	6	(0.77–2.31)	1.25 gm. %
Cholesterol	25	(90–230)	130 mg. %
Cholesterol esters	25	(60–220)	65 mg. %
Potassium	6	(3.7–4.2)	4.0 mEq./L.
Sodium	6	(137–141)	140 mEq./L.
Calcium	6	(4.5–4.8)	4.7 mEq./L.
Chloride	6	(99–109)	103 mEq./L.
Phosphorus	6	(4.9–5.1)	5.0 mg. %

EXPERIMENTAL

Aim of the investigation

The aim of the investigation was to study the relationship between dietary fat and chyle fat, to arrive at conclusions on the absorption of fats in man, and to test these conclusions against the current theories on the mechanism of fat absorption.

Arrangement of the experiments

On the strength of animal experiments and based on Munk's investigations on a patient with a chylous fistula, it is to be expected that a great deal of the fat, under normal circumstances, is absorbed with the chyle. It is, therefore, possible to study the fat absorption, at any rate that via the lymph, by comparison of the composition of chyle fat with that of dietary fat. In contrast to former investigations, it is now possible to compare the composition of the chyle fat as a whole with that of the dietary fat, by means of separate quantitative determinations of all fatty acids. As not all intestinal chyle accumulates in the patient's thorax, absolute measurement of the absorption via the lymph was not possible.

The natural fats, which are mixed triglycerides being composed of widely varying proportions of the fatty acids, n-octanoic to n-docosanoic, were administered to the patient in such a way that each time certain particular fatty acids could be studied. When the patient was given butterfat, the emphasis fell on the short-chain fatty acids, n-tetranoic, n-hexanoic, and n-octanoic; when given margarine and coconut oil, n-octanoic, n-decanoic, and n-dodecanoic acid were preponderant. A synthetic fat, "capryl olive oil," was administered to study the behavior of n-octanoic acid more closely; cacao-butter was given to examine that of n-hexadecanoic and n-octadecanoic acid; and finally a synthetic fat with hydrogenated herring oil was given to investigate the absorption of n-eicosanoic and n-docosanoic acid.

The next step was to study the absorption via the lymph of some *unsaturated fatty acids*. By feeding sunflower oil and linseed oil the absorption of $\Delta^{9,12}$-octadecadienoic acid and $\Delta^{9,12,15}$-octadecatrienoic acid was investigated, while the behavior of Δ^{13}-docosenoic acid was studied by the administration of rapeseed oil. Comparison of the lymphatic absorption of olive oil and eläidinized olive oil enables us to investigate the absorption of Δ^{9}-octadecenoic acid (both cis- and trans-isomers).

As the lymph was aspirated only once a week, the possibility could not be excluded that some fatty acids might be absorbed from the thoracic cavity to a greater extent than others. This is, however, unlikely because the fatty acids are almost completely present as fat in the lymph. A differentiation is therefore impossible here and a specific absorption can therefore not be expected.

It is possible that part of the fat is absorbed via the portal system and the liver. This was not investigated in the case concerned. The possibility should not be overlooked that some fat escapes absorption and is excreted in the feces. In order to keep a check on this the fat balance was always determined by the method of van de Kamer (29).

The patient was kept on a diet with constant amounts of fats, proteins, and carbohydrates during most of the experiments. The daily diet consisted of:

200 ml. protein milk [1]	75 gm. green vegetable
20 gm. rice flour	25 gm. lean meat
20 gm. sugar	16 gm. fat
400 ml. water	75 gm. banana
50 gm. potato	50 ml. orange juice
100 ml. tomato juice	

Vitamins A, D, B-complex [2] and C were added to this diet. Stabilized ferrous chloride was also given per os. The amount of fat was 28 gm. per day; exceptions were made during some periods in which larger or smaller amounts were given. It was necessary during the last two months to increase the amounts of protein and carbohydrate in order to meet the increasing caloric demands.

The type of fat in the diet was changed every week, or group of weeks, by giving a protein milk and fat containing different kinds of glycerides, such as coconut oil or olive oil instead of butterfat.

The chyle was collected by pleural puncture at the end of each week, and subsequently the pleural cavity was examined radiologically for the presence of chylous residues. Because of the possibility that a slight residue may influence the composition of the chyle during the following dietary period, a diet containing one kind of fat was usually given for two successive weeks. The composition of the chyle fat during the second week was considered the most representative.

Methods

The chemical investigation was carried out as follows: All saturated fatty acids with the exception of n-tetranoic acid were quantitatively determined separately. Of the unsaturated fatty acids, only the poly-unsaturated ones could be determined individually by measurement of the absorption spectrum in ultraviolet light after alkaline isomerization. It was impossible to determine the mono-unsaturated fatty acids directly, either separately or *in toto*. The sum of the unsaturated fatty acids was computed by subtracting the total of the saturated fatty acids from the total of all fatty acids. The iodine number also lent support to this calculation. With these restrictions the fatty acid composition of each dietary fat could be compared with that of the corresponding chyle fat. The extraction and analyses of the fat from chyle, feces and blood were carried out by the methods of van de Kamer and his colleagues (30), using the analytical method of Boldingh (25, 26). A mixture of the analytically pure saturated fatty acids, n-hexanoic to n-docosanoic, which contains of each fatty acid 2 to 8 mg., can be analyzed with this method so that each acid is estimated with an accuracy of about 5 per cent. The minimum amount which can be determined with this accuracy is 0.5 to 1.0 mg., depending on the molecular weight.

RESULTS

The chyle fat during a low-fat diet

The chyle fat does not give an accurate impression of the intestinal lymph fat because the chyle consists of a mixture of intestinal and non-intestinal lymph. This should be kept in mind when a comparison is made of the fatty acid composition of chyle fat with that of dietary fat. In other words, if the chyle fats are to be compared with the corresponding dietary fats, a correction must be made for the admixture with non-intestinal lymph fat. The composition of the fat in the non-intestinal lymph is unknown, but it may be assumed that chyle fat collected during a low-fat period is similar to non-intestinal lymph fat, because the fat in the chyle during such a low-fat diet can not be of intestinal origin. The correction factor was determined by analyzing the chyle fat of four low-fat periods. Both the fatty acid composition and the fat content of this chyle were practically constant in all the periods. From this it follows that the composition of the "corrected chyle fat" can be calculated by subtracting the non-intestinal fat from the chyle fat as found by analysis. Then the fatty acid pattern of the "corrected chyle fat" can be compared directly with that of the dietary fat.

The patient was on a low-fat diet for four successive periods of a week each, the amounts of

[1] The protein milk was prepared by Nutricia, Ltd., of Zoetermeer, The Netherlands, and consisted of a base of buttermilk in which 5.5 gm. per 100 ml. butterfat, coconut oil, olive oil, etc. were emulsified without the addition of an emulsifier. In some periods the fat was emulsified in skimmed milk.

[2] One tablet of Vitamin B-complex daily was administered, containing: thiamin 5 mg., riboflavin 2 mg., nicotinic acid amide 20 mg., adermin 2 mg., and calcium pantothenate 3 mg.

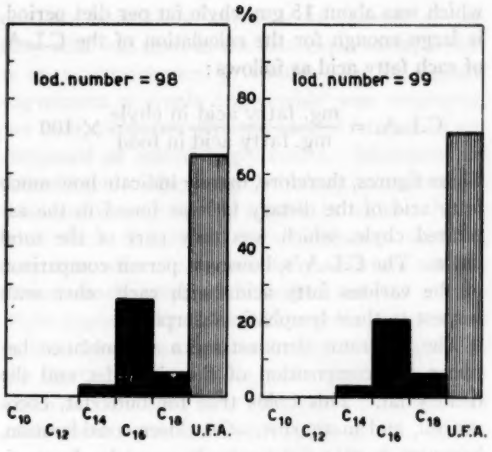

FIG. 1. LEFT: COMPOSITION OF THE PATIENT'S CHYLE FAT DURING A LOW-FAT DIET (LESS THAN 1 GM. FAT DAILY FOR FOUR WEEKS). RIGHT: COMPOSITION OF THE BLOOD FAT OF THE FASTING PATIENT (DURING A DIET CONTAINING RAPESEED OIL)

Solid columns = saturated fatty acids.
Striped columns = unsaturated fatty acids (U.F.A.)

fat given being less than 1 gm. daily. The chyle, which was of a milky white color during a regimen with normal quantities of fat (28 gm. daily), became transparent during the low-fat diet; in appearance it resembled blood plasma. The fat content of the chyle fell markedly, from 3.7 to about 0.6 gm. per cent.

Figure 1 shows the composition of the chyle fat during a low-fat regimen. For purposes of comparison the fatty acid pattern of the blood fat of the fasting patient during another dietary period is also given. There is a striking resemblance between the two fatty acid patterns (31). This does not imply that the forms in which these fatty acids occur (triglycerides, phospholipids, and cholesterol esters) are also similar. Table II shows that during the first period of the low-fat diet there was still an admixture with "high-fat" chyle from the preceding margarine period. In the three following periods, however, the chyle was characterized by a constant low fat content and a constant fatty acid composition. The iodine number of this chyle fat was as high as that of the fat of the blood serum of the fasting patient (see Figure 1).

Table II also shows the composition of the chyle fat of a patient with chyloascites from the Hospital for Sick Children, Great Ormond Street, London. The chyle of this nine-month-old patient was collected during a low-fat diet and was put at our disposal by Drs. W. W. Payne and A. Norman. It appears from these data that the fatty acid pattern of the chyle from their patient shows a resemblance to that of the "low-fat" chyle from ours (see Table II).

The chyle fat during a diet containing butterfat, coconut oil or margarine

The dietary fats and the chyle fats were analyzed in the usual manner, while the n-tetranoic acid content was determined separately by the method of Fairbairn and Harpur (32). The results are shown in Figure 2. The figures on the left show the percentage composition of the dietary fat in comparison with the "corrected" composition of the chyle fat. Those on the right give curves of the lymphatic absorption coefficient of each fatty acid of the dietary fat. These coefficients were cal-

TABLE II

The per cent of fatty acids in the fat from the chylothorax and chyloascites of our patient and the London patient, respectively

Fatty acid	Chylothorax patient					Chyloascites patient
		Low-fat diet				
	Margarine diet 28 gm. per day	I	II	III	IV	Low-fat diet
n-Decanoic (C_{10})	1	—	—	—	—	—
n-Dodecanoic (C_{12})	10	7	<1	1	0	1
n-Tetradecanoic (C_{14})	9	7	3	2	2	2
n-Hexadecanoic (C_{16})	19	21	26	26	28	25
n-Octadecanoic (C_{18})	6	6	6	6	7	10
n-Eicosanoic (C_{20})	—	—	—	—	—	1
Total unsaturated fatty acids	55	59	64	66	63	61
Iodine number	64	77	98	98	89	58
Total fat content of chyle, in per cent	3.70	1.22	0.54	0.52	0.58	0.21
Coefficient of fat absorption, in per cent	96	—	—	—	—	—

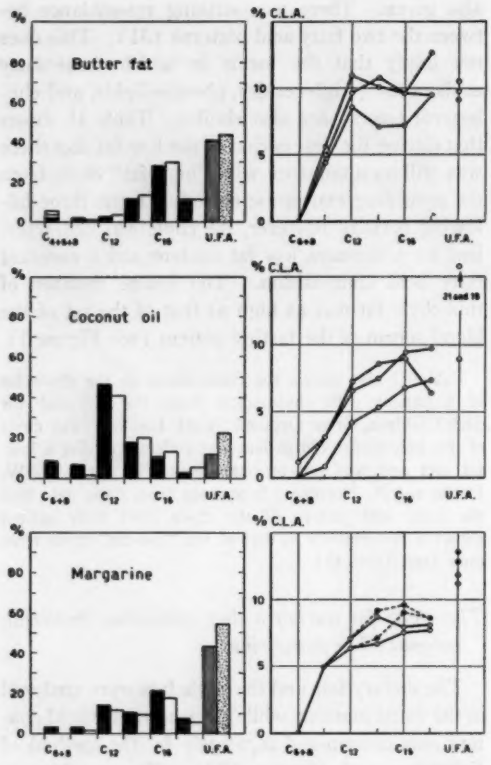

FIG. 2. LEFT: COMPOSITION OF DIETARY FAT AND OF CHYLE FAT (CORRECTED) DURING DIETS CONTAINING RESPECTIVELY BUTTER FAT, COCONUT OIL AND MARGARINE. RIGHT: COEFFICIENTS OF LYMPHATIC ABSORPTION (C.L.A.) OF THE SATURATED AND UNSATURATED FATTY ACIDS

Solid columns = saturated fatty acids in dietary fat.
Open columns = saturated fatty acids in chyle fat.
Striped columns = unsaturated fatty acids in dietary fat.
Stippled columns = unsaturated fatty acids in chyle fat.
○——○ = "corrected values."
●- - - - - - - - ● = "uncorrected values."

culated by determining the percentage of each fatty acid of the dietary fat which was recovered in the chyle. This percentage is called the "coefficient of lymphatic absorption" (C.L.A.) of the fatty acid. We are, of course, quite aware that these absorption coefficients do not indicate the total absorption of a given fatty acid via the lymph because the drainage of chyle from the patient was only partial. Only about 10 per cent of the fat from the food is recovered as chyle fat. This quantity, which was about 15 gm. chyle fat per diet period, is large enough for the calculation of the C.L.A. of each fatty acid as follows:

$$\text{C.L.A.} = \frac{\text{mg. fatty acid in chyle}}{\text{mg. fatty acid in food}} \times 100$$

These figures, therefore, merely indicate how much fatty acid of the dietary fat was found in the aspirated chyle, which was only part of the total chyle. The C.L.A.'s, however, permit comparison of the various fatty acids with each other with respect to their lymphatic absorption.

The diagrams demonstrate a resemblance between the composition of the chyle fat and the dietary fat. This holds true for butterfat, coconut oil, and margarine. On closer consideration, however, certain differences become clearly manifest when the various C.L.A.'s are taken into account (see the graph on the right). n-Tetranoic, n-hexanoic and n-octanoic acid are completely absent in the chyle; the C.L.A. of n-decanoic acid is 3 to 5 per cent; of n-dodecanoic acid 7 to 10 per cent; of n-tetradecanoic acid somewhat more, just as for n-hexadecanoic and n-octadecanoic acid. In this way it is demonstrated that the C.L.A. increases when the chain length of the fatty acids increases. The unsaturated fatty acids are absorbed as well as or somewhat better than the saturated n-tetradecanoic, n-hexadecanoic, and n-octadecanoic acids.

The chyle fat during a diet containing "capryl olive oil"

As it was a very striking feature that the short-chain fatty acids, n-tetranoic, n-hexanoic, and n-octanoic, were not found in the chyle when the patient was given margarine, butter, or coconut oil, it was of importance to verify this observation by administering one of these fatty acids in a greater amount in the form of triglyceride. The following data gathered from the literature are given for purposes of comparison. Kiyasu, Bloom, and Chaikoff (33) administered to rats 1-C^{14}-labelled n-decanoic acid dissolved in maize oil. The lymphatic absorption of this free fatty acid was only 7 to 19 per cent. Frazer used tributyrine (the triglyceride of n-tetranoic acid) which was not found in the chyle after administration to rats. One should, however, be cautious in drawing con-

clusions from these experiments because a free fatty acid was used in the first experiment, which is an unphysiological procedure. In the second experiment a single triglyceride was employed, not to be compared with the natural fats which are composed of mixed triglycerides. Moreover, in contrast to the other long chain fatty acids, n-tetranoic acid is very readily soluble in water. Apart from these objections the experiments show at most that some short-chain fatty acids are not absorbed in the chyle or absorbed only to a very slight degree. We observed the same phenomenon in the absorption of mixed triglycerides in which the short-chain fatty acids, n-tetranoic, n-hexanoic and n-octanoic acid, from margarine, butterfat, and coconut-oil were also absent in the chyle. At the same time, however, the long-chain fatty acids were demonstrated in the chyle. This can only be explained by hydrolysis of the dietary fat.

In the experiment described below, a fat was chosen which would clearly show whether or not hydrolysis occurred. Use was made of "capryl olive oil," a fat containing 46 per cent n-octanoic acid. This was prepared by subjecting a mixture of equal parts of olive oil and tricapryline, the triglyceride of n-octanoic acid, to alkaline re-esterification. In this way a mixed triglyceride was formed in which each molecule contained at least one n-octanoic acid group.[3] This "capryl olive oil" was given to the patient in the usual quantities (28 gm. daily) for one week. The fat balance was normal during this period (absorption coefficient 98 per cent). The fat content of the chyle also retained a normal value (2.1 per cent). This proves that the fat absorption in general, as well as the lymphatic absorption in particular, was normal. For these reasons this experiment with a synthetic fat may be considered a physiological one.

Figure 3 shows that the chyle fat contained only 4 per cent n-octanoic acid in contrast to the dietary fat which contained 46 per cent of this acid. The C.L.A. of n-octanoic acid is only 0.70 per cent, while that of Δ^9-octadecanoic acid is 8.6 per cent. These experiments therefore confirm the findings

[3] The composition of the "capryl olive oil" was calculated as: 27.5 mol. per cent tricapryline; 44.3 mol. per cent "dicapryl olive oil;" 23.9 mol. per cent "monocapryl olive oil;" and 4.3 mol. per cent olive oil.

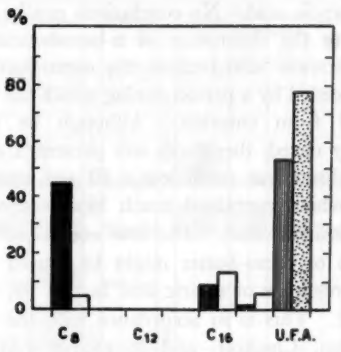

FIG. 3. COMPOSITION OF DIETARY FAT AND OF CHYLE FAT (CORRECTED) DURING A DIET CONTAINING "CAPRYL OLIVE OIL"

Solid columns = saturated fatty acids in dietary fat.
Open columns = saturated fatty acids in chyle fat.
Striped columns = unsaturated fatty acids in dietary fat.
Stippled columns = unsaturated fatty acids in chyle fat.

when butterfat, coconut oil, and margarine are used.

As n-octanoic acid was found in the chyle in only slight amounts, while the "capryl olive oil" was well absorbed (the fat balance was normal), it seemed acceptable that this fatty acid was absorbed via the portal vein or converted in the intestinal wall ($<$ 12.5 mg. per cent octanoic acid). Whether n-octanoic acid could be demonstrated in the blood was therefore investigated. Three and one-half hours after an oral dose of 28 gm. "capryl olive oil" (*i.e.*, the whole amount for one day) venepuncture was carried out and the blood fat analyzed. n-Octanoic acid could not be demonstrated in it, and it may therefore be concluded that n-octanoic acid does not pass the liver as such but is converted in the intestine or liver.

The chyle fat during a diet containing cacao-butter and hydrogenated herring oil with peanut oil

In order to study in more detail the lymphatic absorption of the saturated fatty acids, n-hexadecanoic, n-octadecanoic, n-eicosanoic and n-docosanoic, experiments were carried out with cacao-butter, a fat containing 25 per cent n-hexadecanoic and 35 per cent n-octadecanoic acid, and with a hydrogenated herring oil (re-esterified with peanut oil so that the melting point was 42°C.), a fat containing 8 per cent n-eicosanoic and 8 per cent

n-docosanoic acid. No conclusions can be drawn regarding the absorption of n-hexadecanoic and n-octadecanoic acid because the cacao-butter diet was preceded by a period during which the patient suffered from enteritis. Although he seemed clinically cured, there was still present a steatorrhea (absorption coefficient = 80 per cent), the fat of which contained much hexadecanoic and n-octadecanoic acid. The low coefficient of absorption of cacao-butter might be caused by the high percentage of stearic acid in this fat, *viz.* 35 per cent. This is in accordance with the results of Bloom, Chaikoff, and Reinhardt (23) who found that only 36 to 54 per cent of stearic acid, as free fatty acid dissolved in corn oil, is absorbed by the rat, in contrast to at least 80 per cent of shorter chain fatty acids.

It can only be deduced that n-eicosanoic (C_{20}) and n-docosanoic (C_{22}) acids are absorbed through the lymph. It was, however, impossible to calculate the degree of this absorption because the chyle production had fallen markedly during this period, owing to the patient's spontaneous recovery.

The chyle fat during diets containing fats with unsaturated fatty acids

In order to study more closely the absorption of various unsaturated fatty acids, Δ^9-octadecenoic acid (cis and trans-isomers), $\Delta^{9,12}$-octadecadienoic acid, $\Delta^{9,12,15}$-octadecatrienoic acid, and Δ^{13}-docosenoic acid, experiments were carried out with olive oil (containing cis-Δ^9-octadecenoic acid), eläidinized olive oil (containing trans-Δ^9-octadecenoic acid), sunflower seed oil (containing $\Delta^{9,12}$-octadecadienoic acid and $\Delta^{9,12,15}$-octadecatrienoic acid), linseed oil (*idem*) and rapeseed oil + peanut oil (containing Δ^{13}-docosenoic acid). The results are represented in Figure 4. In all cases there was a great degree of resemblance between the composition of chyle fat and dietary fat.

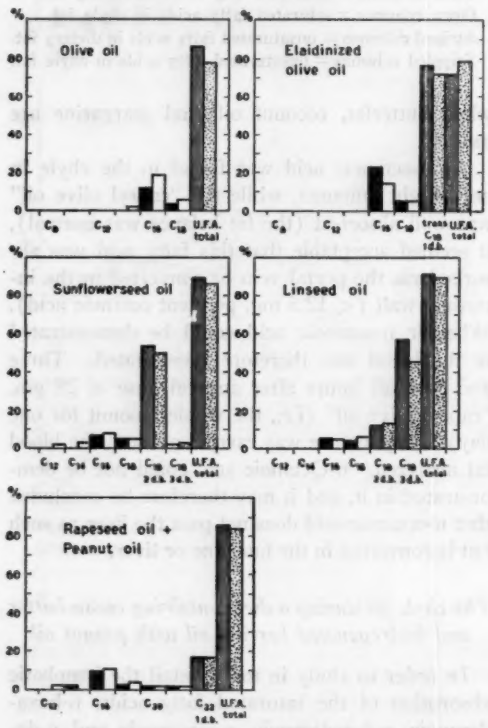

FIG. 4. COMPOSITION OF DIETARY FAT AND OF CHYLE FAT (CORRECTED) DURING DIETS CONTAINING FATS WITH UNSATURATED FATTY ACIDS

Solid columns = saturated fatty acids in dietary fat.
Open columns = saturated fatty acids in chyle fat.
Striped columns = unsaturated fatty acids in dietary fat.
Stippled columns = unsaturated fatty acids in chyle fat.

Summary

The results of the entire investigation are comprised in Figure 5. The composition of the chyle fat remained practically unchanged in successive periods when the same dietary fat was given. On the other hand, when a change was made in the dietary fat a simultaneous change in the chyle fat was observed. The similarities and differences between the dietary fat and the corresponding chyle fat are also evident in the Figure.

DISCUSSION

Experiments have indicated that the fatty acid pattern of the chyle fat differs from that of the dietary fat.

Of the *saturated fatty acids*, n-tetranoic and n-hexanoic are not present in the lymph, while n-octanoic is found only to a very slight degree, even if this fatty acid is present in large amounts in the dietary fat. n-Decanoic acid is found in the lymph but its absorption is less than that of n-dodecanoic, n-tetradecanoic, n-hexadecanoic, and

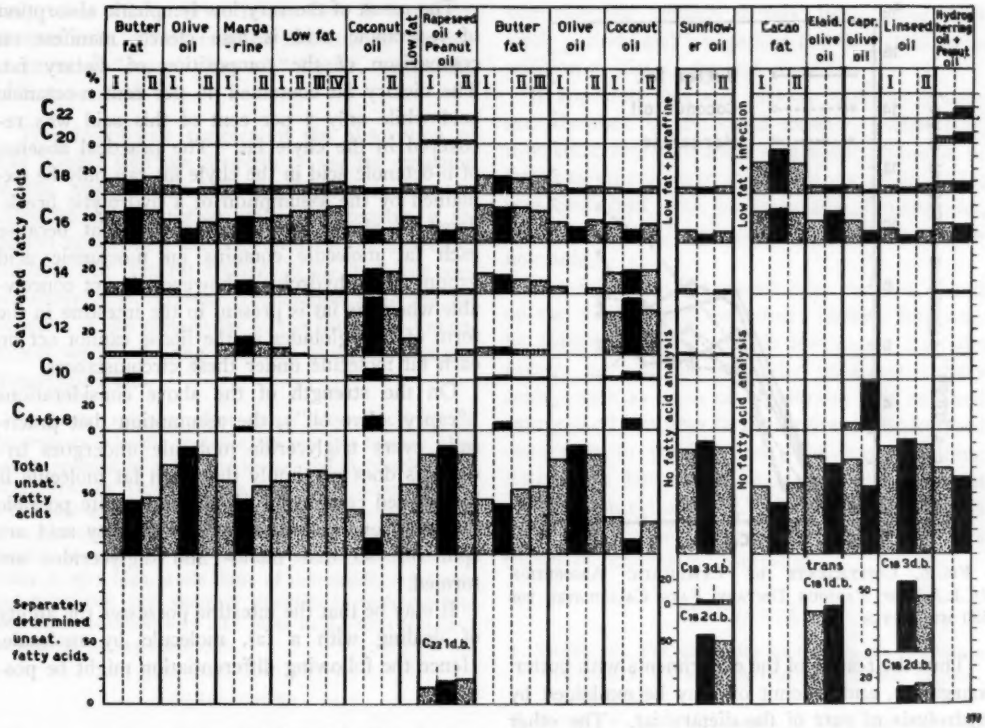

FIG. 5. COMPARISON OF THE FATTY ACIDS OF THE DIETARY FAT WITH THE FATTY ACIDS OF THE CHYLE, UNCORRECTED VALUES, REPRESENTED IN CHRONOLOGICAL ORDER DURING ALL DIETS OF THE WHOLE PERIOD OF OBSERVATION

Solid columns = fatty acids of diet.
Stippled columns = fatty acids of chyle.

n-octadecanoic. The four last-mentioned fatty acids are absorbed practically to the same extent. This can be deduced from Figure 6 which shows the coefficients of lymphatic absorption (C.L.A.) of the saturated fatty acids of various fats. The lines run closely parallel in the same direction which proves clearly that the C.L.A.'s of n-hexanoic, n-octanoic, n-decanoic, and n-dodecanoic acid increase as the carbon chains become longer.

In order to compare the C.L.A.'s of the fatty acids in the various periods, it is necessary to eliminate the differences in the production of chyle during these periods (see Figure 7). Although some objections may be raised to this procedure, the fatty acids found in the chyle were recalculated for a standard chyle production of 500 ml. per week. This seems to be justifiable since the fat content of the chyle was independent of the quantity of chyle produced per week and also because the amount of chyle was independent of the nature of the dietary fat. The diminution of the quantities of chyle aspirated weekly was due to the patient's spontaneous recovery.

The various *unsaturated fatty acids* were absorbed well. The C.L.A.'s of these fatty acids are of the same order as those of the saturated fatty acids, n-tetradecanoic, n-hexadecanoic, and n-octadecanoic.

It follows from the difference in lymphatic absorption of the saturated fatty acids, n-octanoic, n-decanoic, and n-dodecanoic, that the dietary fat must be hydrolyzed at least partially during absorption. Otherwise, if dietary fat was absorbed without hydrolysis, there would be no difference in the absorption coefficient of the three mentioned fatty acids.

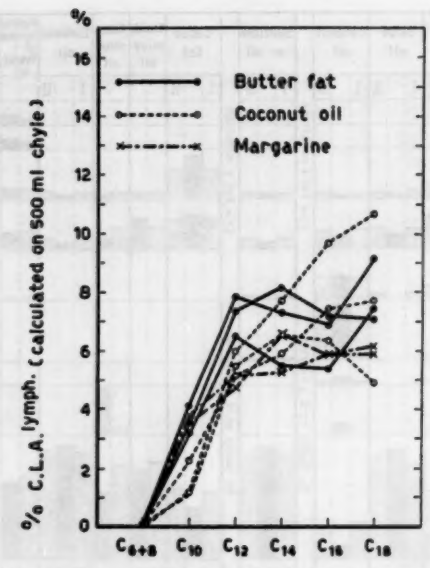

FIG. 6. COEFFICIENTS OF LYMPHATIC ABSORPTION (C.L.A.) OF VARIOUS DIETARY FATS CALCULATED FOR 500 ML. CHYLE

Thus the results of the experiments with butter, margarine, and coconut oil may be explained by hydrolysis of *part* of the dietary fat. The other part of it might pass through the intestinal wall as triglyceride and be absorbed with the chyle. In the experiment with "capryl olive oil" almost every molecule of fat must have been hydrolyzed, as Figure 5 shows that the C.L.A. of n-octanoic acid is only 0.7 per cent, while the coefficient for the longer chain fatty acids of this fat amounts to 8.6 per cent.

The result of the very low lymphatic absorption of n-octanoic acid is also clearly manifest on comparison of the composition of dietary fat. The dietary fat contained 46 per cent n-octanoic acid while only 4 per cent of this acid was recovered in the chyle fat. The practical absence of n-octanoic acid in the chyle fat can only be explained by the assumption of a hydrolytic breakdown of almost every molecule of fat because each fat molecule contains an n-octanoic acid group. This hydrolysis, however, is not conceivable when the fat is present in the intestine in the form of tiny globules as the lipase cannot act on each fat molecule under these circumstances.

On the strength of the above considerations ("capryl olive oil"), the assumption that practically every triglyceride molecule undergoes hydrolysis does not imply that each fat molecule is hydrolyzed completely (1–2); it is quite possible that only one or two molecules of fatty acid are split off, so that mono- and diglycerides are formed.

It may be that the intestine possesses the ability of dealing with a fat, molecule by molecule. Hence the following differentiation might be possible:

1. Certain fats might be absorbed almost without hydrolysis, *e.g.*, the greater part of the markedly unsaturated fats, olive oil, sunflower seed oil, linseed oil, and rapeseed oil.

2. Other fats must first be hydrolyzed completely or partially, *viz.* the fats containing short-chain fatty acids. After resynthesis, transport to the chyle follows, but the liberated short-chain

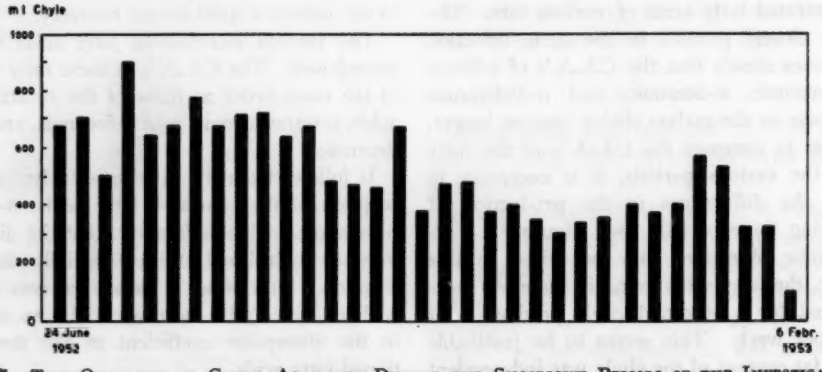

FIG. 7. THE QUANTITY OF CHYLE ASPIRATED DURING THE SUCCESSIVE PERIODS OF THE INVESTIGATION

fatty acids are converted in the liver or in the intestinal cells. This may be the case in the absorption of "capryl olive oil," butter, margarine, and coconut oil.

This hypothesis concerning the absorption of fats implies that the droplets of the fat emulsion in the intestine (3–12) disintegrate into molecules during absorption, with, if necessary, a selection of the fatty acids of which these molecules are built up. The fat droplets in the chyle are consequently of a different composition from the fat droplets of the dietary fat in the intestinal lumen, unless we are dealing with the absorption of the unsaturated fats.

A selective splitting off of special fatty acids from the *fat droplets* is hardly probable, the more so because these are surrounded by a layer of mono- and diglycerides (3–12). Frazer's view that the fatty acids n-tetranoic to n-decanoic are removed selectively from the fat droplets after hydrolysis, owing to their solubility in water, is debatable, because only the solubility of n-tetranoic acid in water is very high, while those of n-hexanoic, n-octanoic and n-decanoic acid decrease successively with rapidity. The above selection may take place in the intestinal contents or in the intestinal cells.

CONCLUSIONS

It may be stated that the nature of a dietary fat decides whether or not it is hydrolyzed in the intestinal lumen and subsequently partially resynthesized to chyle fat in the intestinal wall. If there is a hydrolysis followed by resynthesis, these processes are such that the degree of lymphatic absorption is different for each fatty acid. This means that the absorption mechanism is accompanied by considerable selective capacity. Selection of triglycerides or fatty acids, however, is only possible if the fat droplets, each actually consisting of millions of molecules, disintegrate.

On the strength of this idea it is conceivable that some dietary fats (olive oil, sunflower seed oil, etc.) pass more or less unchanged into the chyle, and that in the case of other fats (butter, coconut oil, margarine, etc.) the fatty acid pattern of the chyle fat differs considerably from that of the dietary fat.

SUMMARY

The absorption of fat via the lymph was studied in an otherwise healthy child with chylothorax by administering various dietary fats during two-week periods and comparing the fatty acid composition of dietary fat and chyle fat.

It appeared that the various fatty acids were not absorbed to the same extent. n-Hexanoic, n-octanoic and n-decanoic acid were not found, or found to only a slight degree, in the chyle. This was confirmed with an especially prepared fat, "capryl olive oil," containing 46 per cent n-octanoic acid.

The following hypothesis was evolved, based on the experiments described: Certain fats may be absorbed almost without hydrolysis, *e.g.*, the greater part of the markedly unsaturated fatty acids (such as olive oil, sunflower seed oil, and rapeseed oil). Other fats must first be hydrolyzed completely or partially, *viz.*, the fats containing short-chain fatty acids, such as butter, coconut oil, and "capryl olive oil." In the latter case this hypothesis cannot be reconciled with Frazer's theory of particulate absorption.

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OBSERVATIONS UPON THE WITHDRAWAL OF SODIUM CHLORIDE FROM THE DIET IN HYPERTENSIVE AND NORMOTENSIVE INDIVIDUALS

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The mechanism by which the kidney conserves sodium and chloride in the face of a suddenly diminished dietary intake has been the subject of much investigation (1–4). The possible importance of defining this mechanism more adequately was emphasized by the claim that in hypertensive subjects the response to dietary salt withdrawal differs from the response in normal subjects. Perera and Blood (5) suggested that the kidneys of hypertensive subjects are capable of retaining salt more efficiently than the kidneys of individuals with normal blood pressures (either as a result of primary renal changes or of changes mediated by the adrenal cortex), and this has been interpreted as evidence that the adrenal cortex in hypertensive subjects is abnormal with regard to the hormone or hormones which influence the renal tubular reabsorption of salt (6). We have attempted to detect an adrenal response to a decrease in sodium intake and the results are reported elsewhere (7). This provided an opportunity for re-investigating, in normotensive and hypertensive subjects, other aspects of the response to sudden and almost complete withdrawal of sodium chloride from the diet. In view of the figures reported by Perera and Blood (5), observations were made on the change in body weight and volume of urine excreted in the first twenty-four-hour periods after salt withdrawal. Changes in the urinary excretion of sodium, potassium, chloride, and nitrogen under these conditions were estimated. A few estimations of the fecal excretion of sodium, potassium, and nitrogen were also carried out.

Subjects

Six normotensive and six hypertensive hospital patients were used in this study. The former included four patients who had been admitted for relatively trivial complaints (*i.e.*, hemorrhoids, mild upper respiratory infection, dyspepsia, prolapse of intervertebral disc producing mild sciatica) and who had recovered prior to the period of investigation, and two subjects who were not suffering from any disease, and in whom there was no known family history of hypertension. The relevant clinical data of these subjects are given in Table I with similar data for the hypertensive cases. These latter subjects were patients in whom essential hypertension had been found either incidentally in the course of a general physical examination or who had been referred to hospital for insomnia or headaches. All subjects were within the range for ideal weight as given by the Metropolitan Life Insurance Company. No evidence of heart failure or other disease process was present in any subject and renal function, as tested by the capacity to dilute and concentrate urine maximally and by estimation of glomerular filtration rate using the clearance of inulin or creatinine, was within normal limits. Protein was not detected in the urine of any subject and the blood urea nitrogen was normal in all cases. The ages of both the hypertensive and normotensive groups were comparable and the sex distribution was similar.

Diet

All subjects were fed a completely constant diet throughout the period of investigation. This con-

TABLE I

Relevant clinical data

Case No.	Age *Yrs.*	Sex	Mean blood pressure with range of daily observations taken over 7 to 10 days during period with salt supplement: Systolic *mm. Hg*	Diastolic *mm. Hg*
			Hypertensive subjects	
1	49	F	220 ± 15	128 ± 13
2	43	F	205 ± 15	110 ± 5
3	40	M	152 ± 8	110 ± 7
4	43	M	169 ± 10	103 ± 8
5	40	F	160 ± 10	108 ± 5
6	46	M	167 ± 13	105 ± 10
			Normal subjects	
1	54	M	110 ± 5	65 ± 5
2	43	F	137 ± 8	87 ± 5
3	35	F	115 ± 0	68 ± 2
4	50	M	118 ± 5	67 ± 5
5	47	F	130 ± 5	80 ± 5
6	34	M	120 ± 5	75 ± 5

sisted of measured amounts of rice, sugar, salt-free bread, salt-free butter, jam, fruit and fruit juice, tea, drinking water, and one egg daily. Additional protein was provided by giving a fixed amount of Casilan (Glaxo) which is virtually sodium and chloride free. The total amount of food consumed differed from subject to subject according to the weight and size but was constant for each subject from day to day and its caloric value ranged from 1500 to 2500. Thirty to fifty grams of protein was provided by the Casilan and the total protein content of the diet ranged from 50 to 70 gm. per day. The calories in the diet were adequate to maintain weight and all subjects were in nitrogen balance. The daily intake of potassium in such a diet is almost entirely dependent upon the amount and nature of fruit and fruit juice consumed. In order that this should remain constant it was found necessary to keep the type of fruit eaten by the patient the same throughout, and a given batch of canned juice was used for each patient. Batch differences are responsible for variations in the intake of potassium between patients and in three cases in which the intake was low, additional amounts of potassium were given by adding fixed amounts of potassium citrate to the juice (hypertensive case No. 6 and normotensive cases No. 4 and 6). The sodium content of the diet was 8 mEq. per day.

Plan of experiments

During the course of the experiments all subjects were allowed out of bed for toilet purposes and for a few hours in the afternoons. The diet was supplemented by capsules of sodium chloride, 3 to 6 gm. of which was eaten throughout each day. This was continued until the excretion of the electrolytes under study in the urine became stabilized, *i.e.*, after 4 to 5 days of the diet. The capsules were omitted from the diet after a stable control period of from 7 to 14 days, the patient continuing to eat the diet until the end of the experiment. Twenty-four-hour collections of urine were analyzed and blood was withdrawn at intervals for analysis. In four cases analysis of fecal electrolytes was done.

Methods

Serum, urinary and fecal sodium and potassium were measured in a Barclay Flame Photometer using lithium as an internal standard. Chloride was estimated by Van Slyke's method (8) and total urinary nitrogen by the micro-Kjeldahl method. Subjects were weighed in a beam balance accurate to 50 gm. prior to breakfast and after micturition.

RESULTS

1. *Body weight and urine volume*

During the control period of 7 to 14 days the body weight of all subjects showed small day-to-day variations without any systematic trend in either direction. Changes in body weight after 24 and 48 hours of withdrawal of sodium chloride from the diet of both normal and hypertensive subjects are shown in Table II. The change in the urine volume during the first 24 hours after salt restriction is also shown. The variation in each group is considerable and the data overlap to a large extent in the two groups. The absolute fall in weight for the normal subjects ranges from 0.10 to 0.60 Kg. (0.13 to 0.83 per cent of body weight) and for hypertensive subjects the weight loss ranges from 0 to 0.35 Kg. (0 to 0.43 per cent body weight). The mean changes are not significantly different ($P = 0.2$). Relatively comparable and slightly larger losses were observed after 48 hours of salt restriction. Alterations in the volume of urine excreted in the 24 hours after salt restriction are also shown in Table II. The average changes in the two groups, + 242 ml. and + 227 ml., are not significantly different ($P = 0.9$).

TABLE II

Changes in body weight, Kg., and in urine volume, ml., 24 and 48 hours after salt restriction

	Normotensive subjects				Hypertensive subjects			
	Absolute body weight change *Kg.*		% Wt. change	Change in urine volume *ml.*	Absolute body weight change *Kg.*		% Wt. change	Change in urine volume *ml.*
Subject	24 hrs.	48 hrs.	24 hrs.	24 hrs.	24 hrs.	48 hrs.	24 hrs.	24 hrs.
1	−.25	−.55	−.34	+100	−.10	− .40	−.17	+210
2	−.50	−.80	−.75	− 60	0	− .20	0	+200
3	−.15	−.70	−.36	+310	−.15	− .85	−.26	−160
4	−.10	−.70	−.13	+110	−.10	− .35	−.21	+280
5	−.20	−.70	−.23	+170	−.35	−1.00	−.43	+300
6	−.60	−.10	−.83	+820	−.25	− .30	−.42	+530
Mean	−.30	−.59	−.44	+242	−.16	− .52	−.25	+227

TABLE III

Urinary excretion of sodium

Case No.	Mean 24-hr. urinary excretion Na prior to salt withdrawal *mEq./24 hrs.*	Na in urine in 1st 24 hrs. after salt withdrawal as % of mean 24-hr. excretion in control period	Number of days over which urinary excretion of sodium remained over 10 mEq./day after salt withdrawal *mEq.*	Cumulative negative Na balance after salt withdrawal as a result of continued urinary loss (*more than 10 mEq./day*)
		Hypertensive subjects		
1	95.0	70.5	10	162
2	78.7	97.8	12	202
3	78.2	81.8	8	142
4	48.8	96.3	6	104
5	80.7	43.4	10	178
6	103.6	79.2	7	164
Mean	80.8	78.2	9	159
		Normal subjects		
1	81.8	74.3	9	236
2	79.9	90.1	8	188
3	88.8	63.5	4	61
4	87.5	118.4	5	215
5	99.9	83.2	4	130
6	89.7	86.5	10	396
Mean	87.9	86.0	7	204

2. *Electrolytes*

Urinary sodium: Following the withdrawal of salt from the diet, the amount of sodium and chloride excreted in the urine rapidly diminished over the ensuing few days until it reached a level of below 10 mEq. per day. Thereafter the body was in only slight negative sodium balance as a result of continued small losses of sodium in the feces, sweat, and urine. The response to withdrawal of salt from the diet in the two groups of subjects is compared in Table III, in which consideration is given to three points, namely 1) the amount of sodium remaining in the urine during the first twenty-four hours after withdrawal of salt, 2) the number of days over which the urinary excretion remained above 10 mEq. per day and, 3) the total sodium deficit which developed as a result of continued urinary excretion during the days of marked negative balance following salt withdrawal. In this way the speed (and efficiency) with which the kidney responds to the sudden change in sodium intake is directly compared in the normotensive and hypertensive subjects.

It is seen that the mean urinary excretion of sodium prior to salt withdrawal is comparable in the two groups. Although there is some individual variation, the average figures for the three criteria for the normotensive and hypertensive subjects are not significantly different. After twenty-four hours of salt restriction, the average excretion of sodium in the urine fell to 78 per cent and 86 per cent of the amount excreted in the control period for the hypertensive and normotensive groups, respectively. The twenty-four-hour urinary loss of sodium was reduced to 5 to 10 mEq. in an average of nine days by the hypertensive subjects and in an average of seven days by the normotensive subjects after dietary salt restriction. These figures are not significantly different. The total cumulative negative sodium balance which developed as a result of continued urinary loss in that time averaged 159 mEq. for the hypertensive subjects and 204 mEq. for the normotensive cases. This difference is not statistically significant ($P = 0.3$).

Urinary chloride: Similar and nearly identical figures were obtained from estimations of urinary chloride in both groups and when they were analyzed in a similar way no marked difference was detectable between the two groups. It is of interest that calculations of water loss based upon total chloride deficits and serum concentrations, and assuming the losses of electrolytes to be extracellular, account for 60 to 100 per cent of the body weight loss over the period.

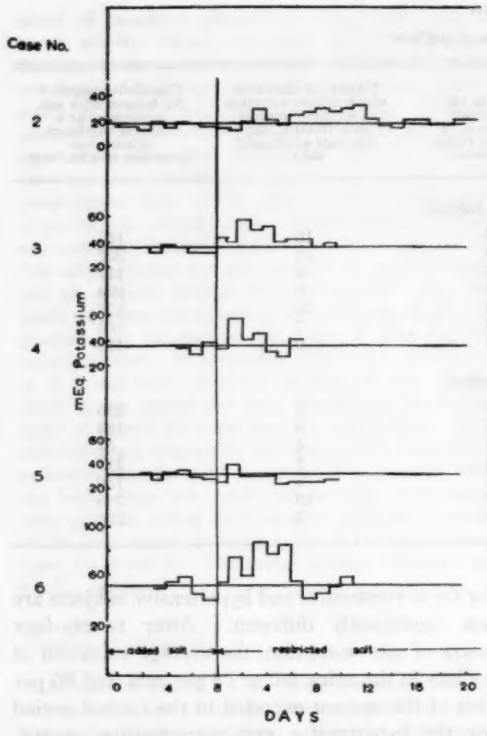

FIG. 1. NORMOTENSIVE SUBJECTS—TWENTY-FOUR-HOUR URINARY EXCRETION OF POTASSIUM PRIOR TO AND FOLLOWING THE WITHDRAWAL OF SALT FROM THE DIET WHICH WAS KEPT CONSTANT IN ALL OTHER RESPECTS

The horizontal line represents the mean excretion during the control period.

Urinary potassium: Following the withdrawal of salt from the diet and during the time when the urinary excretion of sodium was rapidly diminishing, a temporary increase in the excretion of potassium in the urine in both normal and hypertensive subjects was regularly noted. The increase was maximal about the time when the amount of urinary sodium was approaching 10 mEq. per L. but its time of onset and its duration varied from individual to individual. Figures 1 and 2 illustrate these changes in urinary excretion and Table IV provides a summary of the data for five normal and six hypertensive subjects studied in this way. The number of days over which the excess potassium was detected in the urine and the total cumulative increase in urinary potassium, which represents negative potassium balance, are given. Although the variation from case to case is considerable, no subject was observed in whom an increase in urinary potassium was not seen and no significance can be attached to the slight differences in the mean figures for the two groups of cases. The average urinary potassium excretion in both groups is comparable and the average total cumulative potassium loss as a result of increased urinary excretion amounts to 77 mEq. for the hypertensive subjects and 62 mEq. for the normotensive cases.

Serum electrolytes: Repeated estimations of the concentration of serum sodium, chloride, and potassium and of the packed cell volume prior to restriction of sodium chloride and for 30 days after its withdrawal did not reveal significant alterations.

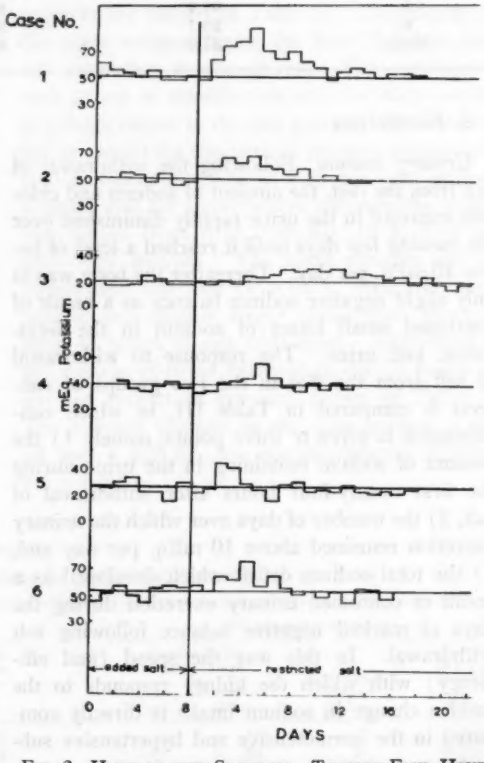

FIG. 2. HYPERTENSIVE SUBJECTS—TWENTY-FOUR-HOUR URINARY EXCRETION OF POTASSIUM PRIOR TO AND FOLLOWING THE WITHDRAWAL OF SALT FROM THE DIET WHICH WAS KEPT CONSTANT IN ALL OTHER RESPECTS

The horizontal line represents the mean excretion during the control period.

TABLE IV

Urinary excretion of potassium

Case No.	Mean 24-hour K excretion prior to salt withdrawal *mEq./24 hrs.*	Total cumulative K loss during negative K balance after salt withdrawal *mEq.*	Days after salt withdrawal during which negative K balance occurred
	Hypertensive subjects		
1	51	162	2nd–10th
2	20	84	5th–18th
3	52	86	1st – 9th
4	39	33	3rd – 7th
5	28	43	3rd – 4th
6	52	56	1st – 7th
Mean	40	77	2nd– 9th
	Normal subjects		
1	—	—	—
2	18	60	7th–12th
3	36	94	1st – 9th
4	36	37	2nd– 4th
5	31	8	2nd
6	52	113	1st – 6th
Mean	35	62	3rd – 7th

Fecal electrolytes: The fecal excretion of sodium, potassium, and chloride was determined in only four cases for a few days in the control period and after salt restriction. The excretion by this route was small in relation to the urinary losses and as might be expected, was not influenced by salt restriction.

Nitrogen: No consistent or significant alteration in the urinary excretion of nitrogen in the urine was observed throughout the control period or during the phase of negative sodium or potassium balance in any of the twelve subjects. The day-to-day variation in nitrogen excretion in any individual case was small and any appreciable change related to the alterations in electrolyte balance could have been detected. The significance of this constancy is discussed in relation to the alterations in potassium balance below.

DISCUSSION

The results obtained by Perera and Blood (5) appeared to establish the existence of a very clear difference in the response of normotensive and hypertensive subjects to salt withdrawal. This difference took the form of a significantly smaller fall in weight on the part of the hypertensives in the first twenty-four hours after withdrawal of salt from the diet and was associated with a smaller total twenty-four-hour urinary volume. It seemed likely that such differences in weight occurring acutely after salt withdrawal were due to alteration in water and salt content of the body, and Perera and Blood stated that "calculation of sodium and chloride clearances in two control subjects and two hypertensive patients showed little change in the non-hypertensive individual following salt restriction but a definite reduction in clearance values in patients with elevated blood pressure." Our results show that there is considerable individual variation in the weight and urinary volume changes during the first twenty-four and forty-eight hours after the withdrawal of salt but that there is no consistent difference between the normotensive and hypertensive groups. This occurred in spite of the rigid control of dietary intake and uniformity of the conditions under which the experiments were conducted. It is possible that the number of cases studied from this point of view is too small to demonstrate any slight difference that might exist between such groups. The results obtained by Thomas, Howard, and Isaacs (9) with 83 medical students suggest that this might be the case, though their results are difficult to interpret because of the absence of control hypertensive subjects of comparable age. It is reasonable to conclude, however, from both our own results and those of Thomas, Howard, and Isaacs that any difference in response, if it exists at all, is slight.

The response in relation to the immediate reduction in the urinary excretion of sodium chloride, the total sodium chloride deficit and the number of days required to establish near sodium balance in the two groups is a more reliable demonstration of the close similarity in behaviour in hypertensive and normotensive subjects. These observations over several days are likely to be more accurate than total body weight changes in twenty-four hours, and if the latter possess any physiological significance it is likely to be revealed in such observations. The similarity in the response as judged by these studies lends no support to the view that the hypertensive subject reacts very differently from the normal. In view of the fact that the serum sodium and chloride concentrations show no detectable alteration within the first 14 days of salt restriction, the present data do not support the idea that the re-

nal clearance of sodium or chloride is significantly different in the two groups, as suggested by Perera and Blood, on the basis of studies in two cases. In addition, and contrary to the observations of these workers, none of our subjects complained of symptoms suggestive of salt depletion throughout, the entire experimental period. A possible explanation of the differences between our results and those of Perera and Blood may perhaps be found in our more rigid control of salt intake before the period of salt deprivation. We found that the urinary excretion of electrolytes and nitrogen became stable after a period of 4 to 5 days of the diet and a further control period of 7 to 14 days was then undertaken before withdrawing salt. The cases studied by Perera and Blood were given the experimental diet for only two days prior to salt restriction. It is evident from the data of these authors that only five out of eleven subjects in which it is possible to make any estimate of balance were, in point of fact, in chloride balance on the day prior to salt withdrawal. Three out of four of the normal subjects excreted chloride in the urine considerably in excess of estimated intake while three out of seven hypertensive subjects excreted considerably less chloride in the urine than was ingested, no hypertensive subject apparently being in negative balance. Furthermore, the published data are not consistent with the statement that no change in the clearance of chloride occurs in the hypertensive subjects since, in the absence of any change in serum concentrations, constancy of clearance would only result from constancy in chloride excretion after salt restriction, and the data do not show such constancy.

The dangers of interpreting observed differences in the rate of salt or water excretion or in body weight changes in terms of abnormal adrenocortical activity are considerable. It is possible that, among other factors, minimal and sub-clinical degrees of cardiac insufficiency might alter the response to salt withdrawal in ambulant subjects in the direction observed by Perera and Blood. On the other hand, it is difficult to visualize factors operating to obliterate any such difference if it has physiological reality.

The existence of a period of negative potassium balance following the withdrawal of salt from the diet has been the subject of controversy and its existence would afford circumstantial evidence of an adrenal response. Black, Platt, and Stanbury (1) were unable to confirm the presence of an increase in urinary potassium to a level higher than the intake, such as was noted by Leaf and Couter (3) in three hypertensive subjects following salt withdrawal, and they considered this to be evidence against the view that a DOC-like substance acted to conserve salt under such conditions. The magnitude of the potassium loss, however, in the subjects we have investigated is such that its existence would escape notice unless there was rigid control of potassium and nitrogen intake throughout the control and experimental period. It seems likely that the failure to observe this loss in the experiments of Black, Platt, and Stanbury is due to the fact that the patients were in negative nitrogen balance and that their consumption of the modified rice diet was admittedly not kept rigidly constant. Although, in our own experiments, there was some variation in the duration and magnitude of the increased urinary potassium loss, it was greatest where the urinary sodium loss was approaching minimal values and when the subject had nearly re-established sodium balance. After this the urinary potassium loss subsided and the subjects returned to the state of potassium balance. Since no increase in the urinary excretion of nitrogen was detected during the phase of increased potassium loss in the urine the latter cannot be ascribed to body tissue breakdown. Estimations of the rate of glomerular filtration carried out at intervals before and after salt restriction in those subjects have not been reported in detail for they do not help to solve the problem of the mechanism of salt conservation in the face of restricted intake. Taken in conjunction, however, with the evidence for increased tubular reabsorption of salt in such circumstances presented by Black, Platt, and Stanbury, the coincidence of increased potassium urinary loss, unattended by an increase in nitrogen excretion, with marked diminution of sodium and chloride in the urine is consistent with the view that the two phenomena could be due to increased activity or secretion of a salt retaining hormone of the adrenal glands. This view is in agreement with that of Leaf and Couter (3) and has recently received direct support from the work of Luetscher and Johnson on aldosterone (4).

The fact that the increase in the urinary excretion of potassium under these conditions is temporary deserves comment. It does not necessarily mean that the increased adrenocortical activity, suggested as being indicated by this, is temporary for it has been shown in man that the administration of adrenocorticotrophic hormone, cortisone and desoxycorticosterone acetate produces little or no urinary potassium loss when the sodium intake has been restricted (10, 11). Evidence to be presented elsewhere, however, suggests that there is a temporary increase in the excretion of adrenocorticoids roughly coincident with the period of potassium loss (7).

SUMMARY

1. Observations have been made upon the change in body weight, urinary volume and the urinary excretion of sodium, potassium, chloride, and nitrogen in hypertensive and normal subjects following sudden and virtually complete dietary restriction of sodium chloride.

2. During the first 24 and 48 hours of salt deprivation, body weight decreased in all subjects. This decrease was not significantly different in hypertensive and normal subjects.

3. Following the withdrawal of sodium chloride from the diet, the rates at which the urinary excretion of sodium and chloride decreased and the total body deficit of sodium and chloride resulting from continued urinary loss were not significantly different in the two groups of subjects. These observations on a small number of cases support the view that any difference which might be present between the two groups is so small as to be of no significance.

4. After the withdrawal of sodium chloride from the diet and during the time when the urinary excretion of sodium was rapidly diminishing a temporary increase in the excretion of potassium in the urine in hypertensive and normal subjects was regularly noted. This was not associated with an increase in the excretion of urinary nitrogen.

5. It seems reasonable to interpret this observation as indicating an increase in adrenocortical activity in immediate response to withdrawal of salt from the diet.

ACKNOWLEDGMENTS

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THYROID FUNCTION ASSAY WITH RADIOIODINE. III. CLINICAL TEST RESULTS AND DIAGNOSTIC LIMITS FOR RATE CONSTANTS[1]

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In an effort to increase the accuracy of assay of thyroidal function, two earlier papers (1, 2) have given a numerical and mathematical description of the short-time behavior of radioiodine observed in the neck tissue, thyroid, plasma, and urine. It remains: a) to apply these data to the conversion of several of the diagnostic parameters currently in use in this type of investigation so as to achieve a common basis for comparison; b) to describe the authors' clinical results; and c) to provide a set of diagnostic limits for routine use based upon a maximum amount of published information.

CHOICE OF PARAMETERS

The various parameters that are commonly employed in the assay of thyroid function with radioiodine are:

(a) The thyroidal plasma clearance rate (3);
(b) The thyroidal fractional rate of uptake from the iodide pool (1);
(c) The 24-hour percentage accumulation in the thyroid gland;
(d) The 24-hour percentage excreted in the urine;
(e) Parameters based on various other bulk samples of urine (4);
(f) The thyroidal rate of output of organic iodine (5); and
(g) The "conversion ratio" (6) of plasma organic and inorganic radioiodine.

Because the mathematical treatment (1) has been restricted to the early phase of iodine uptake, the resulting equations cannot be applied to the diagnostic parameters (f) and (g) above, because these are derived from the later (hormone and organic iodine) phases.

In comparing the remaining parameters it is necessary to convert them to some common basis, which is conveniently chosen as either the pair of plasma clearance rates—thyroidal (expressed as g ml. per min.) and renal (r ml. per min.)—or the corresponding pair of rate factors—thyroidal (k_1 min.$^{-1}$) and renal (k_2 min.$^{-1}$)—as defined in an earlier paper (1).

It may be recalled that the clearance rates are equal to the corresponding rate factors multiplied by the volume of the iodide space.

Clearance rates may have slightly more fundamental significance than the rate factors, but in practice their measurement is performed in exactly the same manner except for the additional determination of the radioiodine in a sample of blood plasma; unless the latter is done very carefully indeed, this may add further experimental error to the measured clearance rate while little is gained in diagnostic precision.

At first sight it might seem that a simpler basis of comparison would be provided by the per cent uptake (or excretion) at some selected time (usually 24 hours) after the administration of the test dose, because this is one single parameter rather than a pair. It must be remembered, however, that the per cent uptake really involves *both* clearance rates (or both rate factors), because a high thyroidal uptake can result either from a high thyroidal rate combined with a normal renal rate or from a moderate thyroidal rate with a low renal rate. Moreover, the possible excretion of organic radioiodine from the thyroid gland may lower the observed per cent uptake to such a value that a hyperthyroid case may appear in the normal range of values; this makes the per cent uptake a one-sided parameter. The per cent excretion

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also has this disadvantage, and it is of course an even more indirect approach to the problem.

In the next section the above parameters are compared on a numerical basis, using data published by others, for those papers where sufficient information has been provided to allow comparison. The search for information of this type has been fairly extensive but not exhaustive, and some papers may have been unintentionally overlooked. There is, however, a whole group of studies, based on the method of Stanley and Astwood (7), that cannot be adapted because these writers have expressed their results in terms of the square root of the time—a mathematical procedure that cannot be linked up with any of the proposed schemes of iodine metabolism. With this group there is the added difficulty that the published numerical parameters depend largely on the different experimental arrangements employed at various centers.

DIAGNOSTIC LIMITS DEDUCED FROM PUBLISHED WORK

Clearance rates

From equations *I* (4),[3] *I* (8), and *I* (9) of an earlier paper (1), it is seen that the clearance rates are related to the rate factors by the following:

$$k_1 = g/{}_3\nabla_0 \qquad (1)$$

$$k_2 = r/\nabla_{30} \qquad (2)$$

[3] In this paper equations prefixed *I* refer to those given in the first paper (1) in this series.

Additional knowledge is needed of the mean reciprocal volume ($1/\nabla_{30}$ ml.$^{-1}$) of the iodide space for the average patient.

From the information discussed in the same paper (1) the over-all mean value

$$W/V_{30} = 3.25 \text{ kg./litre}$$

and also

$$\nabla_{30} = V_{30}/1.32 \qquad I(21)$$

giving, for an average body-weight W of 70 kg.,

$$\nabla_{30} = \frac{70{,}000}{3.25 \times 1.32} = 16{,}300 \text{ ml.} \qquad (3)$$

Putting this value in equations (1) and (2) enables the conversion to k_1 and k_2 of various published figures for clearance rates, as set out in Table I. Although Berkson, Keating, Power, and McConahey (8, 9) deduced their results indirectly from measurements of the radioiodine excreted in the urine, the mid-point of their overlapping values of g, (the thyroidal clearance rate) with normal and hyperthyroid cases, is in good agreement with that of Myant, Corbett, Honour, and Pochin (3); the wide limits of Ansell, Macgregor, Miller, and Wayne (5) are also consistent with these two. On the above results it is apparent that g = 60 ml. per minute or $10^3k_1 = 3.7$ minute^{-1} is the approximate borderline between euthyroid and hyperthyroid cases with normal renal function.

Perry and Hughes (10) have shown that, when renal function is impaired, the average normal

TABLE I

Conversion of published information on clearance rates

Authors	Reference number	Number of cases	Diagnostic category	Clearance rate *ml./min.* g	Clearance rate *ml./min.* r	Deduced rate factors *minute*$^{-1}$ 10^3k_1	Deduced rate factors *minute*$^{-1}$ 10^3k_2 (mean)
Myant *et al.*	3	12 euthyroid 12 Graves' disease	Mid-point of overlapping values normal/hyperthyroid	63	30	3.87	1.84
McConahey *et al.*	9	9 euthyroid 16 hyperthyroid	Mid-point of overlapping values normal/hyperthyroid	57	33	3.50	2.02
Ansell *et al.*	5	Not stated	Certainly normal Upper limit of normal	40 80		2.46 4.52	
Perry and Hughes	10	11	Mean value of normal thyroid but with renal disease	4.3 ±1.5	5.5 ±1.2	0.26 ±0.09	0.34 ±0.07

thyroid values of g (and k_1) are lowered as are the renal parameters; however the number of patients studied is too small to permit a diagnostic boundary value to be set for this type of patient.

Gross 24-hour uptake

The total neck-tissue-plus-thyroid uptake at 24 hours (11, 12) has been a popular parameter at many centers, and optimistic claims have been made for its diagnostic accuracy, presumably because highly hyperthyroid individuals have been tested and compared with definitely normal cases.

To deduce information on k_1 and k_2 from the 24-hour uptake one must consider equation *I(11)*

$$N = \frac{N_0 V_n}{V} e^{-k\phi t} + (1 - e^{-k\phi t})\, k_1/k \quad I(11)$$

which gives the gross uptake provided no radioiodine has left the thyroid gland in organic form; in other words this equation really gives an upper limit for the gross uptake with given values of k_1 and k_2. Then if X is the diagnostic boundary value for the 24-hour uptake, higher values being taken as hyperthyroid and lower values as euthyroid one can say that if the right hand side of equation *I(11)* is not greater than X, then the gross uptake N also cannot be greater than X so the case would be a normal one. This may be written as an inequality

$$N \leq \frac{N_0 V_n}{V} e^{-k\phi t} + (1 - e^{-k\phi t})\, k_1/k \leq X \quad (4)$$

On the other hand if the formula leads to a quantity greater than X one cannot be sure whether N is greater or less than X so the diagnosis is in doubt. Thus writing

$$\frac{N_0 V_n}{V} e^{-k\phi t} + (1 - e^{-k\phi t})\, k_1/k = X \quad (5)$$

leads to a value of k_1 for any given value of k_2, because $k = k_1 + k_2$. Then if k_1 for any patient is less than this value the patient must be euthyroid. Still there will be a region of doubtful k_1 values just above this boundary. This explains one of the major disadvantages of the 24-hour parameter; another, less critical, difficulty is the uncertainty in the term N_0V_n/V unless it has been determined by experiment for the exact geometrical arrangement used for *in vivo* counting.

In an early paper Werner, Quimby, and Schmidt (12) set a boundary value of 35 per cent (X = 0.35) for the 24-hour uptake to distinguish between euthyroid and hyperthyroid cases. Jaffe and Ottoman (11) published a similar value, and this appears to have been adopted quite widely by other centers, at least in this country. Recently, however, Werner (13) has used a much higher borderline of 50 to 55 per cent, which is in better agreement with the value to be expected when calculated from the other parameters. The ranges of uptakes given by Fields and LeRoy (14) are also more consistent with the higher boundary.

Taking as an example Werner's limit of X = 0.55, and $N_0V_n/V = 0.063$ at $t = 24$ hours—an estimate made from comparative observations on an athyroid patient using a Geiger counter arranged in the manner described by these authors (12)—equation (5) becomes, with $\phi t = 566$ minutes,

$$.063\, e^{-566k} + (1 - e^{-566k})\, k_1/k = 0.55 \quad (6)$$

Pairs of k values can be found to satisfy (6), typical cases being included in Table III and in Figure 1.

Urinary excretion

Methods for studying thyroid activity by means of the urinary excretion of radioiodine fall into

TABLE II

Conversion of data reported by Keating and his associates

Type of case	Number of cases	Average rate factors deduced from published data *minute*$^{-1}$ 10^2k_1	10^2k_2	Range* for uptake rate 10^2k_1 *minute*$^{-1}$
Normal (controls)	4	1.8	3.4	0.5– 3.2
Euthyroid (but unrelated illness)	28	1.8	3.0	1.3– 2.4
Non-toxic goiter	26	2.7	3.4	1.6– 3.7
Toxic goiter	23	3.7	2.3	0.7– 6.7
Exophthalmic goiter	64	9.1	2.0	7.7–10.5
Myxedema	24	0.5	2.8	0– 0.6

* The range tabulated here is the mean value ± twice the standard deviation given in the original paper (15, 16).

TABLE III

Conversion of Werner's (13) 24-hour uptake limit and Fraser and others (4) urinary T-parameter limits

Renal rate 10^3k_2 *minute*$^{-1}$		Deduced values of the thyroid rate 10^3k_1 *minute*$^{-1}$				
		T = 2.1 Very low/ low uptake borderline	T = 2.8 Low/normal uptake borderline	T = 12.8 Normal/ probably high borderline	24-hour gross uptake 55 per cent Werner upper limit of normal	T = 17.4 Probably high/ high borderline
0.1		—	—	0.50	1.34	0.92
0.2		—	—	1.50	1.41	1.98
0.3		—	—	2.08	1.47	2.57
0.4		—	0.09	2.46	1.53	3.03
0.7		0.17	0.57	3.21	1.74	3.82
1	Normal renal range	0.40	0.85	3.63	1.97	4.32
2		0.53	1.04	4.22	2.81	4.95
3		0.20	0.81	4.18	3.80	4.98
4		—	0.22	3.95	4.89	4.70

two main classes, (a) the analytical method with simple exponential excretion curves which has been used extensively by Keating and his associates (15, 16), and (b) the bulk sample methods of which a recent example is that described by Fraser, Hobson, Arnott, and Emery (4).

Keating's method assumes a simple exponential relation, equivalent to putting $\phi = 1$ in the pres-

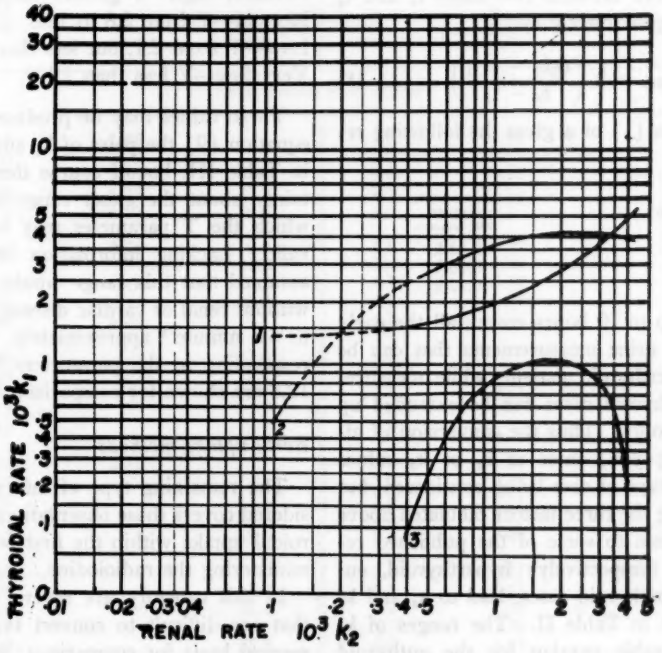

FIG. 1. TYPICAL DIAGNOSTIC BORDERLINES DEDUCED FOR 24-HOUR UPTAKE AND BULK URINE METHODS USING RADIOIODINE

Curve 1—24-hour uptake 55 per cent, reference (13).

Curve 2—Bulk urine method, euthyroid/probably high borderline T = 12.8, reference (4).

Curve 3—As above, low/euthyroid borderline T = 2.8.

ent authors' equations. With this assumption equation *I*(7) may be written

$$kt = -\ln\left(1 - \frac{kE}{k_2}\right) \qquad (7)$$

and, by plotting the observed results for the urine fraction E against the time t in this form on semi-logarithmic paper, a straight line should result. Actually since ϕ does not vary very rapidly with t after the first hour the more precise relation *I*(7) also approximates to a straight line over limited periods of time, and moderate departures from linearity are easily masked by experimental errors in the individual determinations of E. A further masking of these departures can result from the oral rather than the intravenous administration of the test dose, causing "zero" time to be spread out over a period of some minutes.

If equations *I*(7) and (7) are compared it is apparent that the effective average slope of the experimental curve between two times t_1 and t_2 will be given approximately by

$$k_{effective} = k\left(\frac{\phi_2 t_2 - \phi_1 t_1}{t_2 - t_1}\right) \qquad (8)$$

Putting in values (1) of ϕ gives the following results:

Period of observation in hours	Approximate average rate $k_{effective}$
1 to 10	0.45 k
3 to 24	0.36 k
3 to 72	0.32 k

The period up to 10 hours covers all the early useful range of urine measurements that can be made on a hyperthyroid patient, while measurements on hypothyroid cases can be extended up to 72 hours or more. Thus the conversion of effective (Keating-type) rates to k_1 or k_2 values depends on the type of case being considered, but arbitrarily taking the three factors tabulated above and applying them to some of the published results (16) for (respectively) hyperthyroid, euthyroid, and hypothyroid cases, lead to k_1 and k_2 values as shown in Table II. The ranges of k_1 show a considerable overlap for the euthyroid and hyperthyroid groups, possibly caused by breakdown of organically-bound radioiodine during the course of the test in some instances. From the figures tabulated it is difficult to pick a diagnostic boundary with any confidence, but the euthyroid/hyperthyroid border would appear to be somewhere about $10^3k_1 = 3.7$ to 4.2 minute^{-1}, and the euthyroid/hypothyroid border about $10^3k_1 = 0.5$ minute^{-1}. The overall average value of the renal rate is $10^3k_2 = 2.6$ minute^{-1}.

The urinary parameter T taken by Fraser, Hobson, Arnott, and Emery (4) may be represented by

$$T = \frac{\text{Fraction of dose excreted up to 8 hours}}{(\text{Fraction excreted from 8 to 24 hours}) \times (\text{Fraction excreted from 0 to 48 hours})}$$

which, in the present representation, becomes, with ϕ values inserted (1),

$$T = \frac{1 - e^{-247k}}{(e^{-247k} - e^{-866k})(1 - e^{-1003k})\, k_2/k} \qquad (9)$$

Boundary values of T in the original paper (17) are:

High uptake—T greater than 17.4
Probably high—T greater than 12.8, up to 17.4
Normal—T from 2.8 to 12.8
Low—T from 2.1, but less than 2.8
Very low—T less than 2.1

These values may be produced by putting into equation (9) the pairs of k_1 and k_2 values shown in Table III, but of course there is some uncertainty about the exact range of k_2 values over which the T parameter may be expected to be valid. Lacking information on this point it is assumed that this range would extend, for cases without renal or cardiac disease, from $10^3k_2 = 0.4$ to 4.2 minute^{-1} approximately. The curves corresponding to the parameters T = 2.8 and T = 12.8 are shown for comparison in Figure 1.

Early uptake methods

The remaining type of information to be considered covers those observations made of the thyroidal uptake within the first few hours after administering the radioiodine.

In this category are several papers with data that are difficult to convert to the required numerical basis for comparison: thus the results of Reiss, Haigh, Hemphill, Maggs, Reiss, and Smith (17) have been excluded for lack of sufficiently accurate knowledge of their observed neck tissue uptake and other factors; the uptakes of Lowrey, Beierwalters, Lampe, and Gomberg (18) in a

TABLE IV

Results obtained on patients tested by protein-bound iodine and radioiodine methods

PBI μg/100 ml.	Rate factor 10^3k_1 min.$^{-1}$	Combined parameter z	24-Hour uptake %	PBI μg/100 ml.	Rate factor 10^3k_1 min.$^{-1}$	Combined parameter z	24-Hour uptake %
Cases classed as hypothyroid							
0.3			3.1	3.3			17.4
0.5	0		0.8	3.5	0		
0.6	0			3.5	0		
0.6	0			3.6	0.10		
0.7	0.02		3.0	3.6	0		
0.9	0		0.4	3.8	0		15.4
1.0	0		1.7	3.8	0.21		
1.0	0		2.6	3.9	0.18		6.1
1.1	0		14.3	4.4	0	2.10	
1.2	0.17		8.2	4.5	0.16	3.66	
1.8	0.10						
2.0	0.03		1.2	5.2	0.06	3.28	
2.1	0.23		17.6	5.5			3.9
2.5	0.19			5.8	0.08	3.56	12.5
2.7	0.28			6.6			3.3
2.7	0		4.8		0		1.2
2.9	0.10		1.0		0		1.7
2.9	0.18				0		4.0
3.0	0.16		15.2		0.05		2.0
3.2			15.0		0.11		7.7
Cases classed as euthyroid							
2.5	1.15	4.22		4.5	0.81		32.0
2.8	2.30	4.70		4.6	0.65		22.9
3.0	1.71	5.86		4.6	0.21	3.84	15.9
3.2	0.80	4.23	31.2	4.6	0.65		20.0
3.2	0.75	4.19	26.0	4.6	0.25		44.2
3.2	0.40	3.84	19.0	4.6	0.31		17.4
3.2			23.6	4.6	1.90		54.0
3.4	0.65	4.17	33.5	4.7	0.28		17.5
3.8	1.86		43.3	4.7	0.74		41.4
3.9	0.43	4.07		4.7	0.34		
4.0	0.24		13.4	4.7			30.7
4.0	0.58			4.7			12.7
4.0	0.52			4.8	0.71		
4.1	1.18			4.8	1.81		40.6
4.1	0.52			4.9			21.9
4.2	0.22	3.77		5.0	1.56		
4.2	0.75			5.0	0.25		17.0
4.2	1.28			5.0	1.83		10.7
4.2	0.33			5.0	0.32		
4.2	0.55		18.0	5.0	0.49		24.1
4.3	0.62			5.1	0.17	3.83	
4.3	0.29		15.8	5.1	0.45		
4.4	1.10		42.3	5.2	1.40		
4.4	0.75			5.2	1.20		
4.5	0.29		25.7	5.2	1.35		
4.5	0.27		12.7	5.2	0.32		
4.5	0.61		31.4	5.2			17.4
4.5	0.84		31.1	5.2			38.9
4.5	0.67			5.3	0.25		
4.5	0.42		14.5	5.3	0.61		

TABLE IV—*Continued*

PBI μg/100 ml.	Rate factor 10^2k_1 min.$^{-1}$	Combined parameter z	24-Hour uptake %	PBI μg/100 ml.	Rate factor 10^2k_1 min.$^{-1}$	Combined parameter z	24-Hour uptake %
Cases classed as euthyroid—Continued							
5.3	0.65		21.1	5.9	0.46		
5.3	0.95			6.0	0.92		18.7
5.3	0.28			6.0	0.66		
5.4	0.61			6.0	0.28		
5.4	0.56			6.3	1.01		
5.4	0.91			6.4	0.38		18.4
5.5	0.22	4.06	5.4	6.4	0.80		25.0
5.5	0.99		43.0	6.4	0.65		
5.5	0.55			6.5	3.80		
5.5	0.36		23.8	6.5	0.70		
5.5	0.31			6.5			11.1
5.5			30.0	6.8	0.52		21.5
5.6	0.18	3.97	7.8	6.9	3.30		
5.6	1.16		33.9	7.0	1.75		
5.6	0.71		29.1	7.2	0.41		30.0
5.6	0.81			7.2			7.2
5.6	0.68		17.5	7.4	0.57		24.2
5.7	0	2.39	10.3	7.4	0.61		28.0
5.7	2.66			7.5	0	2.74	
5.7	0.59			7.5			22.9
5.7	1.93		41.3	7.6	1.10		22.4
5.8	0.48		21.0	7.8	0.34		15.9
5.8	0.69			7.9	2.08		32.4
5.8	0.34			8.0	0.50		26.2
5.8	0.43			8.0	1.70		37.6
5.9	1.19		31.3	8.2	0.24		
5.9	0.55			9.8	0.71	5.50	
5.9	2.40			10.0			11.1
5.9	1.60			10.1			14.0
5.9	3.40		27.7	13.3	0.73	6.03	36.9
					0.51		26.2
Cases classed as hyperthyroid							
5.8	6.0			10.6	9.8		
5.9	4.1			12.0	11.0		
8.2	4.6			12.2	9.0		
8.3	15.3			13.0	16.0		
9.3	15.8			13.6	17.5		
9.7	7.0						
9.8	20.0			15.4	24.0		
9.9	[illegible]			16.8	14.3		
10.2	16.0			17.2	34.7		
10.4	4.0			17.7	32.6		

small group of children have been omitted for similar reasons; and Crispell, Parson, and Sprinkle (19) have given too meagre details of their counting arrangement.

Apart from the above, there are the Melbourne, Australia, figures of Clarke and Aujard (20) who give a euthyroid/hyperthyroid borderline value of 4.5 to 5.0 per cent for the increase in thyroid uptake between 15 and 25 minutes after injection; since they applied a correction for neck tissue counts, based on thigh counts at frequent intervals, one can express this limit in the present terminology as

$$(e^{-17.7k} - e^{-26.1k})\, k_1/k = 0.0475 \qquad (10)$$

using the values of ϕt presented earlier (1).

TABLE V

Information from early uptake observations

Authors	Number of cases	Diagnostic category	Rate factors in minute^{-1}		
			10^3k_1 range	10^3k_2 range	10^3k_2 mean
Berson, Yalow, Sorrentino, and Roswit (22)	5	Hypothyroid	0.00–0.32	0.70–2.95	2.01
	87	Euthyroid	0.30–3.51	0.88–4.24	2.65
	18	Hyperthyroid	5.5 –38.7	0.42–3.85	2.42
	110	All categories			2.59
This paper	14	Hypothyroid, cardiac		0–0.21	0.04–1.10	0.40
	28	Hypothyroid, non-cardiac		0–0.28	0.08–2.30	1.21
	52	Euthyroid, cardiac		0–1.83	0.02–3.59	0.94
	75	Euthyroid, non-cardiac		0.18–3.80	0.09–3.90	1.53
	1	Hyperthyroid, cardiac		(9.0)	(0.73)	0.73
	22	Hyperthyroid, non-cardiac		4.0–34.7	0.02–4.7	1.82
	67	All categories, cardiac				0.84
	125	All categories, non-cardiac				1.51

For k_2 in the expected range (0.4 to 4.2 × 10^{-3} minute^{-1}) it is easily shown that k_1 needs to be between 6.6 and 7.2 × 10^{-3} minute^{-1} to satisfy this relation.

By a similar line of reasoning, figures given by Kriss (21) for the net uptake in a few patients one hour after injection lead to a euthyroid/hyperthyroid borderline of k_1 ranging from 2.5 to 2.8 × 10^{-3} minute^{-1}; in his paper Kriss makes no mention of the application of any corrections for loss of radioiodine left behind in the injection syringe or for absorption and backscatter in the patient, so the accuracy of these figures is in doubt.

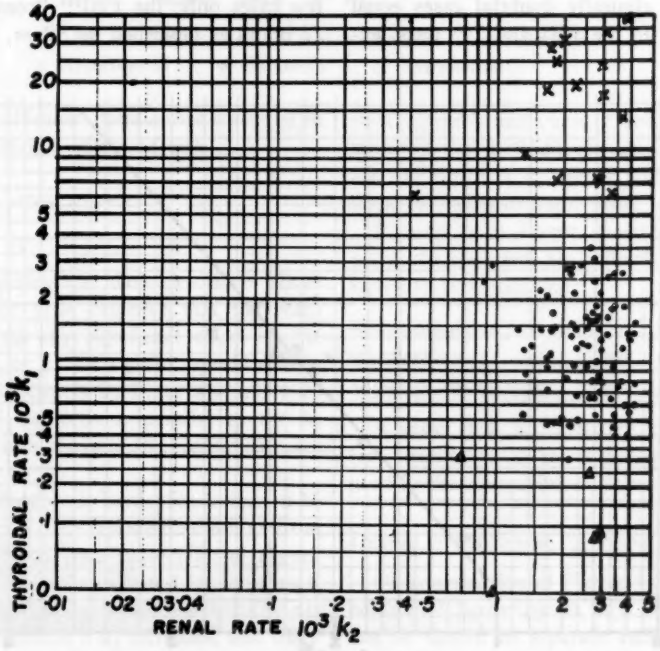

FIG. 2. EXPERIMENTAL RESULTS OF BERSON, YALOW, SORRENTINO, AND ROSWIT

× Hyperthyroid ● Euthyroid △ Hypothyroid

Finally, test results for the New York area are given in the paper by Berson, Yalow, Sorrentino, and Roswit (*22*), nominally in the form of clearance rates calculated assuming a constant ratio of $W/\nabla_{30} = 5.0$ kg./litre; since full data are tabulated these rates are easily converted back to k_1 and k_2 values by using equation *1(4)*, with the results given in Table V and Figure 2.

CLINICAL RESULTS OBTAINED BY THE AUTHORS

Using the methods previously described (*1, 2*), a number of hospital patients have been examined to relate their thyroidal status to the observed rate factor k_1; the results are given in Table IV. The status of each patient was decided independently: (i) on clinical grounds; (ii) on the basis of a protein-bound iodine determination, with tentative borderlines of hypothyroid/euthyroid PBI = 4.0 micrograms iodine per 100 ml. and euthyroid/hyperthyroid PBI = 8.0; and (iii) by the radioiodine test with tentative k_1 limits chosen from the published data discussed above. To decide a few clinically doubtful cases equal weight was given to the PBI and I^{131} tests, expressing the combined diagnostic parameter in the form

$$Z = (PBI)^{\frac{1}{2}} + 1.28 \log_{10} (10^6 k_1) \qquad (11)$$

with diagnostic values of $Z = 3.72$ for the hypothyroid/euthyroid borderline, and $Z = 6.17$ for the euthyroid/hyperthyroid boundary.

This type of parameter was adopted because a plot of the mean experimental values showed an approximate linear correlation between the square root of the PBI and the logarithm of the observed k_1. Figure 3 shows this relation, where each point plotted is the mean of about fifteen observations.

Table V summarizes the numerical values found for k_1 and k_2 for patients in various categories and Figure 4 shows these results in the form of a chart. The low values of the renal rate factor k_2 included in Figure 4 arise mainly from the inclusion of cardiac cases.

In some of the tests additional information has been obtained in the form of the percentage uptake of I^{131} at 24 hours after injection, and, in a few cases only, the PBI^{131} "conversion ratio" at 72 hours as described by Silver, Fieber, and Yo-

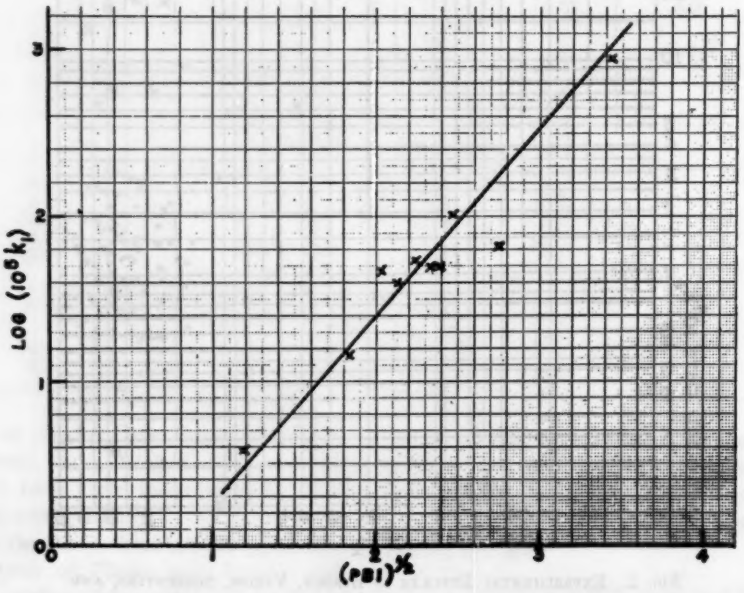

FIG. 3. MEAN EXPERIMENTAL POINTS PLOTTED TO SHOW THE APPROXIMATE RELATION BETWEEN PBI AND RATE FACTOR K_1

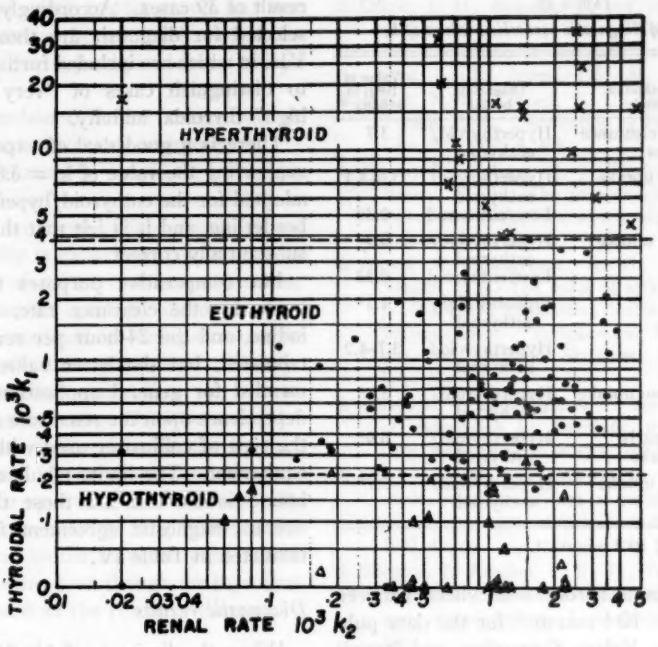

FIG. 4. EXPERIMENTAL RESULTS OBTAINED BY THE AUTHORS
× Hyperthyroid ● Euthyroid △ Hypothyroid

halem (23); the 24-hour uptakes are included in Table IV for purposes of comparison.

DISCUSSION

Renal rate factor k_2

From the experimental data described above there seems to be little evidence that the renal rate (k_2 or r) has any significant effect on the thyroidal rate (k_1 or g) for a given thyroid status; this is shown most clearly by Figures 2 and 4, in which horizontal borderlines, parallel to the k_2 axis, are adequate to separate the euthyroid cases from the other groups.

However, the diagnostic borderline values of k_1 deduced from the 24-hour uptake method, or from the bulk urine method (see Table III and Figure 1), do depend on the value selected for k_2; thus if a constant 24-hour uptake borderline is to be assumed, k_1 must increase if k_2 increases, and there is another *different* relation between k_1 and k_2 if a constant urinary parameter is assumed. Figure 1 illustrates these effects, showing curve 1 concave upwards and curve 2 downwards, and it is apparent that both curves cannot be correct over the full range of k_2 values, and one must choose some particular average value.

If cases with known cardiac or renal disease (10) are excluded, the following mean values of k_2 are to be considered:

Reference number	22	15, 16	9	3	This paper
Number of cases	110	169	25	24	125
Mean value $10^3 k_2$	2.59	2.6	2.02	1.84	1.51

A value of $k_2 = 2.2 \times 10^{-3}$ minute^{-1} has therefore been taken as the weighted mean rate to be used in the conversion of parameters.

Degree of agreement for boundary values of k_1

On examination of Figure 4 it is evident that a horizontal borderline at $k_1 = 3.9 \times 10^{-3}$ minute^{-1} can be drawn to separate euthyroid and hyperthyroid cases, and another line at $k_1 = 0.23 \times 10^{-3}$ minute^{-1} distinguishes (with some over-lapping) between euthyroid and hypothyroid cases. Figure

TABLE VI

Comparison of diagnostic borderline values of k_1

Reference number	Method of study	Diagnostic border	Value of $10^3 k_1$ in minute^{-1}
3, 5, 9	Early clearance rates	Hyperthyroid/euthyroid	3.7
22	Early uptake	Hyperthyroid/euthyroid	3.6–4.1
		Low/euthyroid	0.30
1, 2 and this paper	Early uptake	Hyperthyroid/euthyroid	3.9
		Low/euthyroid	0.23
4	Urine	Probably high/euthyroid	4.2*
15, 16	Urine	Hyperthyroid/euthyroid	3.7–4.2
13	24-Hour uptake	Hyperthyroid/euthyroid	3.0*
20	Very early uptake	Hyperthyroid/euthyroid	6.9*
21	Early uptake	Hyperthyroid/euthyroid	2.7*

* For $k_2 = 2.2 \times 10^{-3}$ minute^{-1}.

2 similarly gives a border somewhere between $k_2 = 3.6$ to 4.1×10^{-3} minute^{-1} for the data published by Berson, Yalow, Sorrentino, and Roswit (22). These results, and the others discussed earlier, are all compared in Table VI. For the hyperthyroid/euthyroid border the mean k_1 from the table is 4.0×10^{-3} minute^{-1}, but for the various reasons given above it is felt that less weight should be given to the last three entries in the table. Moreover, the hypothyroid/euthyroid border of 0.30×10^{-3} minute^{-1} found from Figure 2 is based on only 5 low-uptakes cases, while the value of 0.23×10^{-3} minute^{-1} from Figure 4 is the result of 39 cases. Accordingly, the values finally selected for diagnosis are those given in Table VII, in which are included further limits suggested to distinguish cases of "very low" and "very high" thyroidal activity.

There is a good deal of experimental evidence supporting the value of $k_1 = 3.85 \times 10^{-3}$ minute^{-1} adopted for the euthyroid/hyperthyroid diagnostic borderline, and it is felt that this value should be substantially correct.

For comparative purposes the corresponding limits for the clearance rate, the protein-bound iodine, and the 24-hour per cent uptake are also tabulated, but the latter values are not recommended for general application because of their dependence upon the renal rate factor k_2 and upon the type of collimator used with *in vivo* counting equipment. The limits tabulated for the protein-bound iodine test are those that give the best overall diagnostic agreement for the 174 cases tabulated in Table IV.

Diagnostic results

When the limits in Table VII are applied to the cases listed in Table IV and to a few others for which data have been accumulated from two or more independent tests, the percentage of apparently successful diagnostic results is 87 for the PBI determination, 94 for the k_1 test, and 88 for the 24-hour uptake tests when the latter is applied only to hypothyroid and euthyroid cases.

The few cases in which the PBI^{131} "conversion ratio" was tried were also nearly all euthyroid and low-uptake patients; the apparent success of

TABLE VII

Diagnostic borderline values finally adopted

Parameter	Thyroid borderline status			
	Very low / Low	Low / Euthyroid	Euthyroid / High	High / Very high
Uptake rate 10^3 k_1 minute^{-1}	0.09	0.23	3.85	9.9
Thyroidal plasma clearance rate* g ml./min.	1.5	3.7	63	160
24-Hour uptake per cent†	3.9	8.0	62‡	82‡
Protein-bound iodine§ μg per 100 ml.	2.8	3.9	8.2	10.0
Combined PBI and k_1 (PBI)$^{\frac{1}{4}}$ + 1.28 $\log_{10}$ (10^5 k_1)	2.90	3.72	6.17	7.00

* For $\bar{V}_{10} = 16.3$ litre, see section 3 (a).
† Strictly for the collimator system described (1, 2); these values will not apply for open collimation.
‡ Use of this parameter is not recommended for reasons given in section 3 (b).
§ For best diagnostic results for the cases tabulated in Table IV.

this method (66 per cent) in this group was not very high, but of course better results might be obtained in sorting out hyperthyroid from euthyroid individuals.

Both the protein-bound iodine determination and the measurement of the rate of uptake of I^{131} are seen to give reasonably satisfactory results; in addition the 24-hour per cent uptake of I^{131} is useful particularly for cases of low thyroidal activity where difficulty is experienced in making a complete collection of urine. As is well known, all of these tests can be spoilt by iodine or certain other types of medication, and great care is necessary to exclude this possibility. Finally, therapy with radioiodine may affect the subsequent tests in at least two ways: 1) There may be a residual amount of radioactivity in the thyroid gland, and this must be deducted from the observed amount taken up from the tracer dose; 2) in addition it may take a fairly long time for the stored protein-bound iodine to reach equilibrium at a new low-level value, even though the thyroidal uptake rate is lowered as the result of therapy.

SUMMARY

A series of 180 patients has been examined to determine their thyroidal status by the chemical protein-bound iodine and radioiodine methods, using the thyroidal uptake rate factor calculated from *in vivo* observations at 1, 4, or 24 hours after administration of the tracer dose. Good agreement has been found between the two methods in most cases.

The experimental results, and those of others using some other radioiodine methods, are used to derive suggested diagnostic borderline values of several parameters in common use.

ACKNOWLEDGMENTS

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OBSERVATIONS ON THE MECHANISM OF THE RENAL CLEARANCE OF I^{131} [1]

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Studies of the renal excretion of iodide have been reported from several laboratories in recent years (1–6).[3] Despite this accumulation of information, many properties of the renal mechanisms for excretion of the iodide ion remain unknown. The present study represents an attempt to explore the behavior of the renal clearance of I^{131} under a variety of experimental conditions in human subjects. The clearance of I^{131} has been determined simultaneously with other measurements of renal function and all values, including those for I^{131} clearance, were obtained using a continuous infusion technique. It is assumed that the data derived from the study of I^{131} may be applied freely to explain the behavior of stable inorganic iodide.

METHODS

Case material

The observations are based upon 58 renal clearance studies performed on 49 adult human subjects. Four general groups of patients were included:

1. *Euthyroid subjects with normal renal function:* Thirty-four clearance studies were performed on 31 subjects, all of whom were either convalescent hospitalized patients, or out-patients, without clinical or physiologic evidence of renal disease. The mean age was 33, with a range of 20 to 65 years.

2. *Euthyroid subjects with renal disease:* Thirteen studies were performed on seven patients. Four patients had polycystic renal disease, two chronic glomerulonephritis, and one probable chronic pyelonephritis. Glomerular filtration rates ranged from 9 to 100 cc. per min. per 1.73 sq. m. The mean age was 40 with a range of 23 to 75 years.

3. *Hyperthyroid patients:* The four subjects included in this group ranged in age from 21 years to 46 years and were clinically hyperthyroid at the time of study.

4. *Myxedema patients:* Five patients with primary myxedema and two patients with secondary myxedema were included in this group. The mean age was 40 with a range of 25 to 60 years.

Procedure

Simultaneous determinations of the clearances of inulin, PAH, and I^{131} were performed in all cases. In addition, the excretion rates and plasma levels of sodium, potassium, and chloride were determined during each clearance period. Standard continuous infusion techniques were employed for the measurement of the clearance of inulin and PAH and the continuous infusion method adapted, as previously reported (6), for the determination of the clearance of I^{131}.

All patients were fasting and recumbent at the time of study. In the typical experiment a liter of water was ingested 60 to 90 minutes prior to administration of the priming solution. Subsequent water intake varied with the nature of the experiment. Urine was collected through an inlying multiholed rubber catheter, and urine collections were concluded with two washouts of distilled water, followed by two or more injections of air. Following a timed blank urine collection period, a priming injection of inulin, PAH and I^{131} was administered intravenously and a sustaining infusion of the same substances (in a normal saline vehicle) was delivered by a Bowman constant infusion pump, usually at a rate of 1.5 cc. per min. A 30 to 40-minute period was allowed for equilibration of plasma concentrations, and in the typical experiment, at least three control clearance periods averaging 15 minutes in duration were obtained. When experimental conditions were altered after the control periods, three to seven additional periods were obtained.

Venous blood was sampled at the mid-point of each period through an inlying 17-gauge thin-walled needle, using approximately 0.1 cc. heparin for every 10 cc. of blood. The samples were centrifuged and separated without delay. Inulin was determined according to a modification [4] of the method of Roe, Epstein, and Gold-

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[3] A discussion of the majority of these data is contained in a comprehensive review by Riggs (7).

[4] Modifications include: 1) Heating diluted plasmas and protein precipitating reagents in a 50-degree water bath for 30 minutes after shaking; 2) separation of protein-free filtrates by centrifugation rather than filtra-

stein (8); PAH, according to the method of Smith, Finkelstein, Aliminosa, Crawford, and Graber (9); chloride, according to the method of Van Slyke and Hiller (10); thiocyanate, according to the method of Gregersen and Stewart (11); and sodium and potassium were analyzed on an internally compensated Baird flame photometer. The details of the analytic methods employed in this laboratory for assay of I^{131} in plasma and urine have been reported in a separate communication (6). Donnan corrections were as follows: Na, 0.95; Cl, 1.02; and K, 0.95. I^{131} values were uncorrected.

RESULTS

I. I^{131} *plasma levels*

Continuous infusion of I^{131} throughout experiments, up to but not exceeding three hours in duration, resulted in essentially constant plasma counting rates.[5] Analyses for protein- bound I^{131} were performed in representative cases. The maximum amount of protein-bound I^{131} was observed during the last clearance period in a hyperthyroid patient and was equal to 1.7 per cent of total radio-iodine. In the majority of instances, no protein-binding of infused I^{131} was detected at the conclusion of clearance measurements.

II. I^{131} *clearance as a function of inulin clearance in the steady state*

In Figure 1 the clearance of I^{131} has been plotted against the concurrent value for the clearance of inulin for 40 studies on 34 euthyroid subjects (27 of whom had normal renal function and seven of whom had renal disease) and 11 studies on patients with thyroid dysfunction. All patients had essentially stable filtration rates during the periods of study. The range of glomerular filtration rates for the entire group was from 9 to 155 cc. per min. per 1.73 sq. m. The regression line drawn through the points was derived by the least squares method. The correlation coefficient is 0.82 and the P value less than 0.01, thereby indicating a strong relationship between $C_{I^{131}}$ and C_{In} for group data.

The validity of treating the subjects with normal thyroid function and those with thyroid dysfunction as members of the same population is

tion; 3) treatment of reagent blanks and standards identically with the unknowns; and 4) a wave length of 510 mμ on the Coleman Model 6A Spectrophotometer.

[5] Counting rates ranged from 37.2 to 202.7 cts. per sec. per 2 cc. of plasma. The range for individual subjects $\left(\frac{\text{High} - \text{Low}}{\text{High}} \times 100\right)$ varied from zero to 10 per cent with an average of 4.9 per cent for the group.

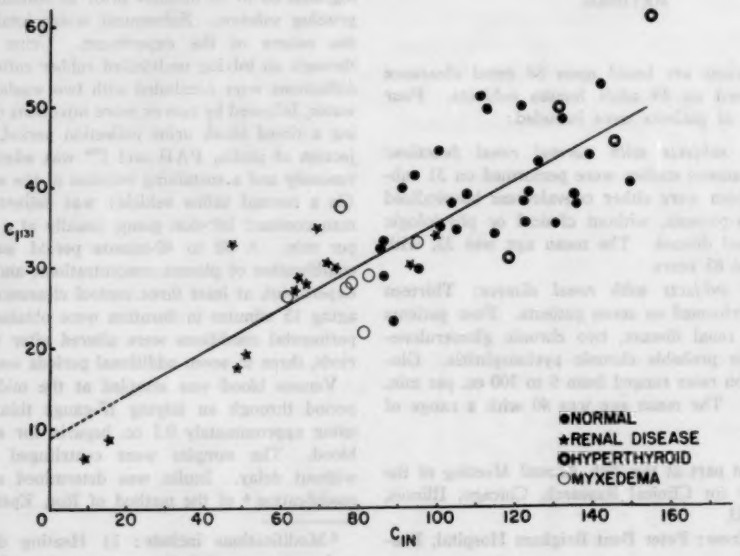

FIG. 1. RELATIONSHIP BETWEEN $C_{I^{131}}$ AND C_{In}

Each point represents the mean value of at least three separate clearance periods. Values are in cc. per min.

based upon a previous observation that a separate plot of $C_{I^{131}}$ *vs.* C_{in} for the present hyperthyroid and myxedematous patients was linear and could not be differentiated statistically from the same plot for euthyroid subjects (6). The equation derived for the combined data of Figure 1 is: $C_{I^{131}} = .268\ C_{in} + 8.6$.

III. $C_{I^{131}}$ *in the presence of acute alterations of glomerular filtration rate*

The linearity noted between glomerular filtration rate and I^{131} clearance in the group plot of patients with stable renal hemodynamics has been tested in individual subjects during acute increases and decreases in GFR.

A. *Acute increases in GFR:* In five subjects, C_{in} was acutely increased by the intravenous administration of Aminophylline. After three control periods, 0.25 to 0.50 gm. of Aminophylline was administered over a 5 to 10-minute period by single intravenous injection and a 10 to 15-minute period was allowed to compensate for delay time. Thereafter from three to five successive clearance periods were obtained. The response in all subjects was qualitatively the same and a representative experiment is recorded in Figure 2. A slight decrease was frequently noted in I^{131} plasma levels following Aminophylline administration; however, in no case did this decrease compensate for the elevation of GFR. Consequently, filtered load of I^{131} invariably rose. In each experiment, the percentage increase in C_{in} was associated with a greater percentage rise in $C_{I^{131}}$ such that the clearance ratios (C_I/C_{in}) consistently increased. Changes in C_{Cl}/C_{in} and C_{Na}/C_{in} were in the same direction as the change for C_I/C_{in}. Values for C_K/C_{in} did not follow a characteristic pattern. C_{PAH} increased slightly in four of the five experiments, but the rise was always less than that of C_{in}. Urine flow increased sharply immediately after injection but the increases were transient and values returned toward control levels after two or three clearance periods.

B. *Acute decreases in GFR:* Acute decreases in glomerular filtration rate were observed in five studies on four patients exhibiting transient peripheral vascular collapse during clearance measurements. One patient had orthostatic hy-

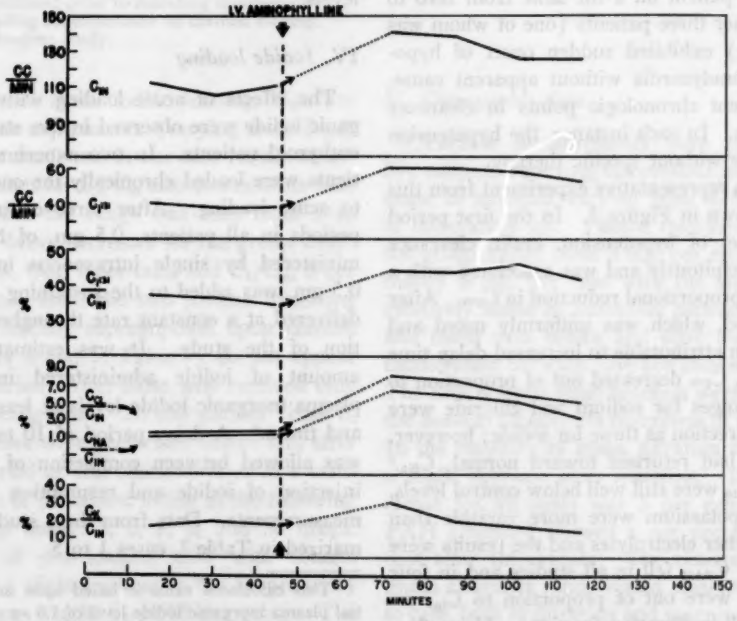

FIG. 2. EFFECTS OF ACUTE CHANGES IN GLOMERULAR FILTRATION RATE
Dotted line represents break for delay period.

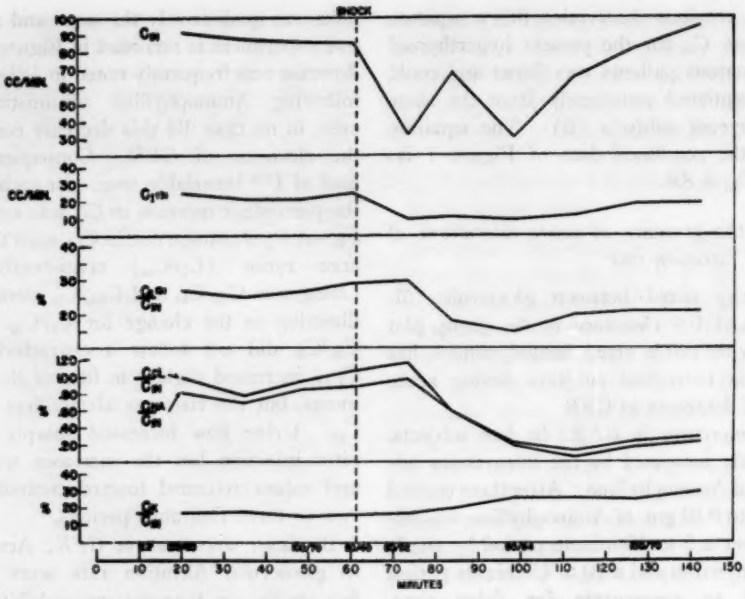

FIG. 3. EFFECTS OF ACUTE CHANGES IN GLOMERULAR FILTRATION RATE

potension and the fall in GFR was induced by elevation of the patient on a tilt table from zero to 90°. The other three patients (one of whom was studied twice) exhibited sudden onset of hypotension and bradycardia without apparent cause, and at different chronologic points in clearance measurements. In each instance, the hypotension was reversible without specific therapy.

Results of a representative experiment from this group are shown in Figure 3. In the first period after the onset of hypotension, inulin clearance decreased precipitously and was associated with a simultaneous proportional reduction in $C_{I^{131}}$. After this lag period, which was uniformly noted and may have been attributable to increased delay time in the nephra, $C_{I^{131}}$ decreased out of proportion to C_{in}. The changes for sodium and chloride were in the same direction as those for iodide; however, when C_I/C_{in} had returned toward normal, C_{Na}/C_{in} and C_{Cl}/C_{in} were still well below control levels. Changes for potassium were more variable than those of the other electrolytes and the results were inconclusive. C_{PAH} fell in all studies and in four the decreases were out of proportion to C_{in}, resulting in a fall in filtration fractions. Urine flow values fell in all instances and generally remained depressed after C_{in} had returned toward control levels.

IV. *Iodide loading*

The effects of acute loading with stable inorganic iodide were observed in five studies on four euthyroid patients. In two experiments, the patients were loaded chronically for one week prior to acute loading. After three control clearance periods in all patients, 0.5 gm. of NaI was administered by single intravenous injection, and 0.5 gm. was added to the sustaining infusion and delivered at a constant rate throughout the duration of the study. It was estimated that the amount of iodide administered increased the plasma inorganic iodide levels at least one thousand times.[6] A delay period of 10 to 15 minutes was allowed between completion of the priming injection of iodide and resumption of clearance measurements. Data from these studies are summarized in Table I, cases 1 to 5.

[6] This calculated value is based upon an assumed initial plasma inorganic iodide level of 1.0 μg per cent, and a maximal volume of distribution of inorganic iodide of 40 per cent of body weight.

TABLE I

The effects of acute and chronic iodide loading on renal hemodynamics, $C_{I^{131}}$, C_I/C_{in}, C_{Na}/C_{in}, C_{Cl}/C_{in}, and C_K/C_{in}

Case		C_{in}* cc./min.	$C_{I^{131}}$* cc./min.	C_I/C_{in} %	C_{PAH}* cc./min.	F.F. %	C_{Na}/C_{in} %	C_{Cl}/C_{in} %	C_K/C_{in} %
1	Control†	101	46	46	536	19	1.7	1.7	22
	Experimental	105	44	43	500	21	2.3	2.5	25
	% Change	+4	− 3	− 7	−7	+12	+31	+47	+14
2	Control	112	50	44	728	15	2.3		28
	Experimental	103	40	38	714	15	2.1		25
	% Change	−8	−21	−14	−2	± 0	− 6		− 9
3	Control	9.3	7.4	80	24	39	15	16	229
	Experimental	8.5	6.0	70	22	38	11	13	239
	% Change	−8	−18	−11	−7	− 2	−29	−23	+ 4
4	Control‡	113	46	41	514	22	1.7	1.9	24
	Experimental	115	46	41	525	22	2.6	2.7	23
	% Change	+1	± 0	± 0	+2	± 0	+53	+44	− 3
5	Control‡	107	34	32	661	16	1.7	1.8	21
	Experimental	108	32	30	676	16	1.5	1.7	17
	% Change	+1	− 4	− 5	+2	− 1	− 8	− 8	−19
	Mean change (1–5)	−2	− 9	− 8	−2	+ 1	+ 5	+15	− 2
6	Control§	108	39	36	510	21	1.3	1.6	18
	Experimental	113	46	41	514	22	1.7	1.9	24
	% Change	+5	+18	+13	+1	+ 4	+30	+20	+31

* Values corrected to 1.73 sq. m. body surface area.
† Control value in all studies is the mean of at least three clearance periods prior to drug administration. Experimental value is the mean of at least three clearance periods subsequent to drug administration. Calculations of % *change* were performed prior to rounding numbers off.
‡ Acute loading superimposed on chronic loading.
§ Chronic loading study.

Subsequent to acute iodide loading, $C_{I^{131}}$ decreased in four experiments (varying from 3 per cent to 21 per cent) and in one case remained unchanged. Values for C_I/C_{in} also decreased in four experiments but the range was only from 5 to 14 per cent. Mean decreases for the group were: C_{in}, 2 per cent, $C_{I^{131}}$, 9 per cent, and C_I/C_{in}, 8 per cent. The data from the two patients receiving acute iodide loading superimposed upon chronic loading (cases 4 and 5, Table I) do not appear to differ materially from those who received only the acute loading dose. In one patient (case 6, Table I) studies were performed before and after a five-day period of oral administration of a saturated solution of potassium iodide in the following stepwise daily doses: 5, 6, 7, 8, and 30 drops. Comparison of post-loading data with control values shows a slight increase in C_{in} (5 per cent) and a moderate rise in $C_{I^{131}}$ (18 per cent). The I^{131}/inulin clearance ratio increased 13 per cent.

V. *Thiocyanate data*

Clearance measurements were performed on five subjects receiving intravenous sodium thiocyanate in amounts varying from 475 to 900 mg. of thiocyanate ion. Plasma thiocyanate levels for the group varied from 3 mg. per cent to approximately 6 mg. per cent with the level remaining relatively stable in individual patients. Diagnosis and mean data before and after thiocyanate administration for each case are shown in Table II. The effects upon renal function were qualitatively the same in all subjects regardless of the diagnosis. In the majority of cases, filtration rate exhibited a slight decrease after injection. The mean decrease for all cases was 5 per cent. Similarly, PAH clearance showed a net decrease of 5 per cent after thiocyanate. The change in the I^{131}/inulin clearance ratios varied somewhat from case to case, both in direction and magnitude. None of the individual changes, however, was marked and

TABLE II

The effects of intravenous sodium thiocyanate on renal hemodynamics, C_I/C_{in}, C_{Na}/C_{in}, C_{Cl}/C_{in}, and C_K/C_{in}

Case		Diagnosis†	P_{SCN}‡ mg. %	C_{in}* cc./min.	C_{PAH}* cc./min.	C_I/C_{in} %	C_{Na}/C_{in} %	C_{Cl}/C_{in} %	C_K/C_{in} %
1	Control§	Eut. norm.	0.0	135	670	30	1.4	1.7	20
	Experimental		3.0	134	684	29	1.5	2.0	20
	% Change			− 1	+ 2	− 5	+ 8	+16	± 0
2	Control	Eut. norm.	0.0	91	559	43	2.2	2.2	
	Experimental		4.5	82	510	46	1.9	1.8	
	% Change			−10	− 9	+ 8	−17	−20	
3	Control	Myx-P	0.0	83	476	35	1.0	1.0	20
	Experimental		3.4	75	400	37	1.2	1.1	20
	% Change			− 9	−16	+ 5	+11	+ 9	± 0
4	Control	Hyp.	0.0	119	548	26	1.1	1.6	19
	Experimental		5.0	121	575	28	1.4	1.9	24
	% Change			+ 2	+ 5	+ 6	+23	+18	+24
5	Control	Hyp.	0.0	155	847	40	0.7	1.2	20
	Experimental		6.0	149	783	35	1.1	1.3	15
	% Change			− 4	− 8	−11	+53	+10	−28
Mean % Change				− 5	− 5	+ 1	+16	+ 6.0	− 1

* Values corrected to 1.73 sq. m. body surface area.
† Eut. norm. = euthyroid subject with normal renal function. Myx-P = primary myxedema. Hyp. = hyperthyroid.
‡ P_{SCN} = plasma thiocyanate levels.
§ Control and experimental as in Table I. Calculations of *% change* were performed prior to rounding numbers off.

the net change for the group was approximately zero. In general, sodium and chloride excretion rates increased after the administration of thiocyanate ion whereas changes in potassium excretion rates were variable. Following thiocyanate administration, plasma levels of I^{131} increased markedly in both hyperthyroid patients and moderately in one euthyroid subject. They remained unaltered in the myxedematous patient and the second euthyroid subject.

VI. *PAH loading data*

Eight patients were included in this group. After control periods, from 60 to 70 cc. of a 20 per cent solution of Na PAH was administered intravenously as a priming solution and the sustaining infusion was calculated to maintain plasma levels at approximately 60 mg. per cent. A period of 45 minutes was allowed for equilibration of plasma levels. Diagnosis and mean clearance values for each case are reproduced in Table III.

Glomerular filtration rates for this group ranged from 49 to 139 cc. per min. In six of the eight cases, filtration rates decreased following PAH loading, (from one to 15 per cent). In seven of the eight cases, the I^{131}/inulin clearance ratios rose. Although the magnitude of the ratio changes was variable, the mean increase for the group was 10 per cent. Sodium/inulin clearance ratios rose in all instances and the mean increase for the group was 291 per cent. Individual responses for chloride and potassium varied; however, the mean changes for both were positive. Thus, chloride/inulin clearance ratios increased 55 per cent, and potassium/inulin clearance ratios increased 30 per cent.

VII. *Mercurial diuretic data*

The effects of intravenous mercurial diuretics were studied in three euthyroid normal subjects. Details of one of these studies are shown graphically in Figure 4. This patient received 2 cc. of Thiomerin® by single intravenous injection over a 10-minute period. Filtration rate declined approximately 15 per cent from control levels subsequent to drug administration and remained relatively constant at the new level. Sodium and chloride excretion rates rose progressively following drug administration. Potassium excretion rate remained relatively stable. I^{131}/inulin clearance ratios showed a tendency to decrease during the 3rd to 5th clearance periods following injec-

TABLE III

The effects of PAH loading on C_{in}, C_I/C_{in}, C_{Na}/C_{in}, C_{Cl}/C_{in}, and C_K/C_{in}

Case		Diagnosis*	C_{in}† cc./min.	C_I/C_{in} %	C_{Na}/C_{in} %	C_{Cl}/C_{in} %	C_K/C_{in} %
1	Control	Eut. norm.	131	38	1.2	1.5	30
	Experimental		131	38	4.0	1.3	26
	% Change‡		0	± 0	+236	− 10	− 14
2	Control	Eut. norm.	139	32	1.7	1.8	24
	Experimental		123	33	4.9	1.7	20
	% Change		−12	+ 3	+187	−5	− 16
3	Control	Eut-RD	74	42	1.5	1.5	30
	Experimental		70	43	5.4	1.6	34
	% Change		− 5	+ 3	+252	+5	+ 13
4	Control	Eut-RD	94	33	1.4	1.3	20
	Experimental		80	40	5.7	1.7	28
	% Change		−15	+22	+308	+ 31	+ 39
5	Control	Eut-RD	49	36	2.1	2.2	22
	Experimental		45	42	6.6	2.9	25
	% Change		− 7	+15	+224	+ 31	+ 16
6	Control	Eut-RD	100	35	0.9	0.7	9
	Experimental		90	39	5.2	0.9	19
	% Change		−10	+12	+452	+ 42	+102
7	Control	Eut-RD	66	43	3.4	2.9	48
	Experimental		65	46	6.9	2.6	35
	% Change		− 1	+ 7	+105	− 12	− 28
8	Control	Eut-RD	65	45	1.3	1.11	29
	Experimental		70	53	8.9	5.09	65
	% Change		+ 8	+19	+563	+359	+125
Mean % Change			− 5	+10	+291	+ 55	+ 30

* Eut-RD = euthyroid patient with renal disease. Other definitions as in preceding tables.
† Values corrected to 1.73 sq. m. body surface area.
‡ Calculations of % *change* were performed prior to rounding numbers off.

tion of the mercurial. Urine flow values rose only during the last two periods of observation. Results in the other two cases were similar except that, 1) GFR did not drop, and 2) values for C_I/C_{in} remained essentially stable.

VIII. *The influence of the excretion of other electrolytes upon $C_{I^{131}}$*

In Figure 5 $C_{I^{131}}$ is plotted against the concurrent values for the clearance of sodium. Plots of C_{Cl} *vs.* $C_{I^{131}}$ and C_K *vs.* $C_{I^{131}}$ are qualitatively the same as that shown for C_{Na} *vs.* $C_{I^{131}}$. Although a rough correlation exists in the group data between $C_{I^{131}}$ and the clearances of the other electrolytes, the relationships are thought to represent an interdependence of each of the variables upon inulin clearance.

Experiments performed in the presence of acute changes of excretion rates of Na, Cl, and K have failed to show changes in $C_{I^{131}}$ which completely parallel any of the former ions. In a group of subjects with pulmonary tuberculosis, it has been noted that following peritoneal puncture with or without induction of pneumoperitoneum, excretion rates of sodium, chloride, and potassium decreased significantly (12). Values for the clearance of iodide, simultaneously determined, were variable and in several patients in whom marked decreases in excretion rates of other electrolytes occurred, iodide clearance was either unchanged or decreased only slightly. A representative study is shown in Figure 6. Other examples of an apparent dissociation of $C_{I^{131}}$ from the excretion of other electrolytes may be seen in the above noted experiments employing mercurial diuretics (Figure 4), PAH loading (Table III), and NaSCN administration (Table II).

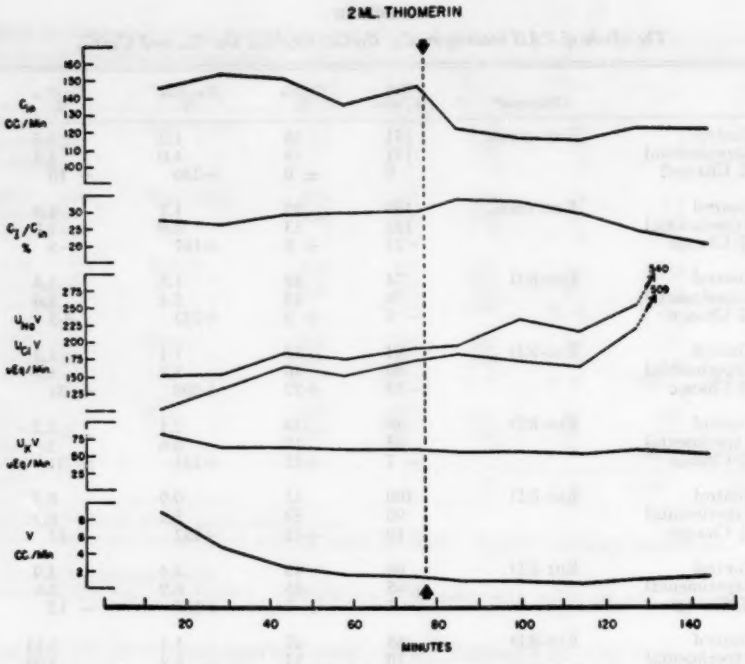

FIG. 4. INTRAVENOUS EFFECTS OF MERCURIAL DIURETICS

$U_{electrolyte}$ V = minute rate of excretion of designated electrolyte.

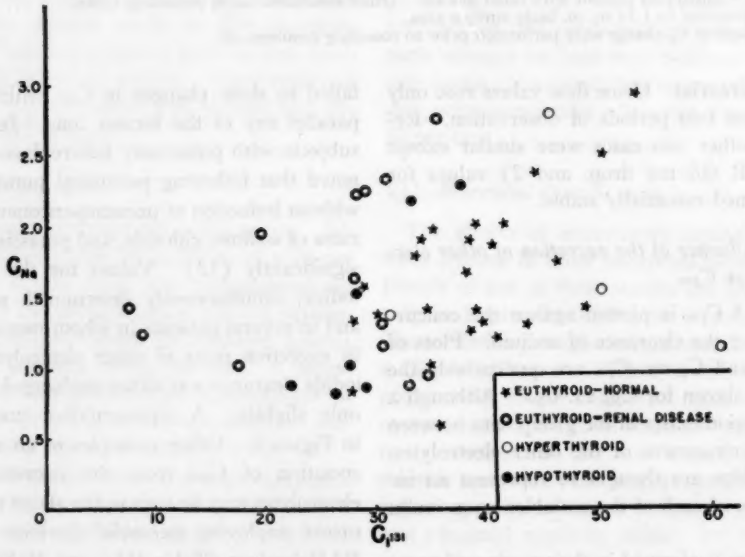

FIG. 5. RELATIONSHIP BETWEEN C_{Na} AND $C_{I^{131}}$

See legend Figure 1.

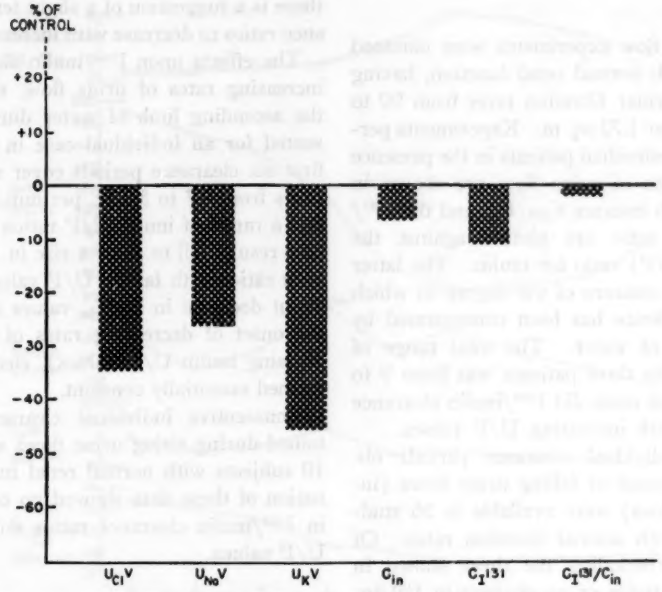

FIG. 6. EFFECTS OF INTRAPERITONEAL AIR INJECTION ON ELECTROLYTE CLEARANCE
See legend Figure 1.

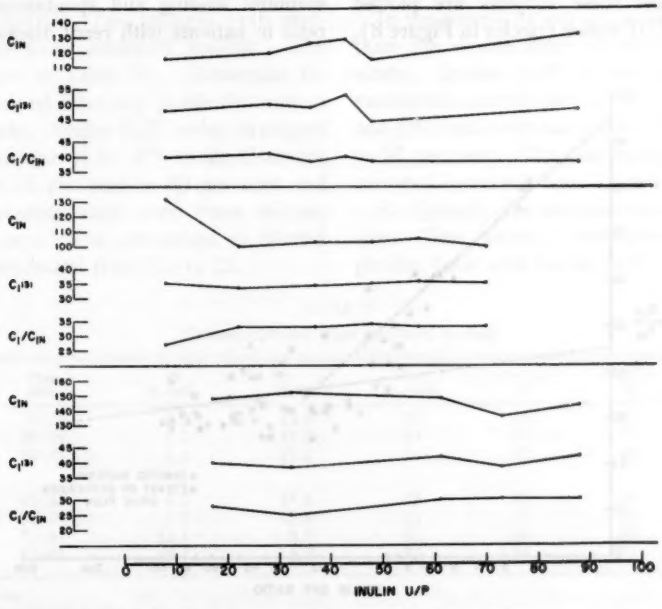

FIG. 7. EFFECTS OF DECREASING URINE FLOW
See legend Figure 1.

IX. *Urine flow*

Data for urine flow experiments were obtained from patients with normal renal function, having a range of glomerular filtration rates from 90 to 153 cc. per min. per 1.73 sq. m. Experiments performed on three individual patients in the presence of decreasing rates of urine flow are shown in Figure 7. In each instance $C_{I^{131}}$, C_{In}, and the I^{131}/inulin clearance ratio are plotted against the urine/plasma (U/P) ratio for inulin. The latter is employed as a measure of the degree to which the glomerular filtrate has been concentrated by the reabsorption of water. The total range of U/P values for the three patients was from 9 to 90. In none of the cases did I^{131}/inulin clearance ratios decrease with increasing U/P values.

Consecutive individual clearance periods obtained during constant or falling urine flows (increasing U/P values) were available in 16 studies on patients with normal filtration rates. Of these, 10 cases (including the three shown in Figure 7) had variable or no changes in I^{131}/inulin clearance ratios, four had decreasing I^{131}/inulin clearance ratios, and two had increasing I^{131}/inulin clearance ratios. When the I^{131}/inulin clearance ratios for these subjects are plotted against the inulin U/P ratios (circles in Figure 8), there is a suggestion of a slight tendency for clearance ratios to decrease with increasing U/P values.

The effects upon I^{131}/inulin clearance ratios of increasing rates of urine flow, measured during the ascending limb of water diuresis, are represented for an individual case in Figure 9. The first six clearance periods cover a range of urine flows from 0.7 to 5.8 cc. per min. per 1.73 sq. m., and a range of inulin U/P ratios from 190 to 20. The results fail to show a rise in I^{131}/inulin clearance ratios with falling U/P values, and in fact a slight decrease in C_I/C_{in} values occurred. With the onset of decreasing rates of urine flow (increasing inulin U/P values), clearance ratios remained essentially constant.

Consecutive individual clearance periods obtained during rising urine flows were available in 10 subjects with normal renal function. Examination of these data showed no consistent change in I^{131}/inulin clearance ratios with falling inulin U/P values.

X. *Osmotic diuresis*

The behavior of $C_{I^{131}}$ has been investigated in the presence of osmotic diuresis associated with mannitol loading and spontaneous osmotic diuresis in patients with renal disease.

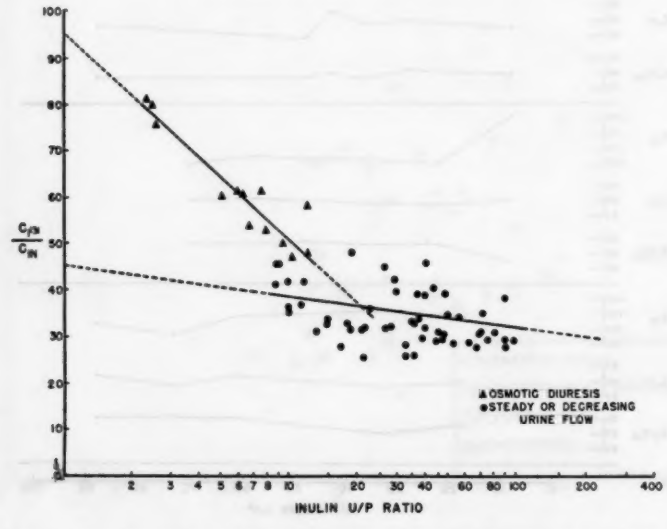

FIG. 8. RELATIONSHIP BETWEEN I^{131}/INULIN CLEARANCE RATIO AND INULIN U/P RATIO

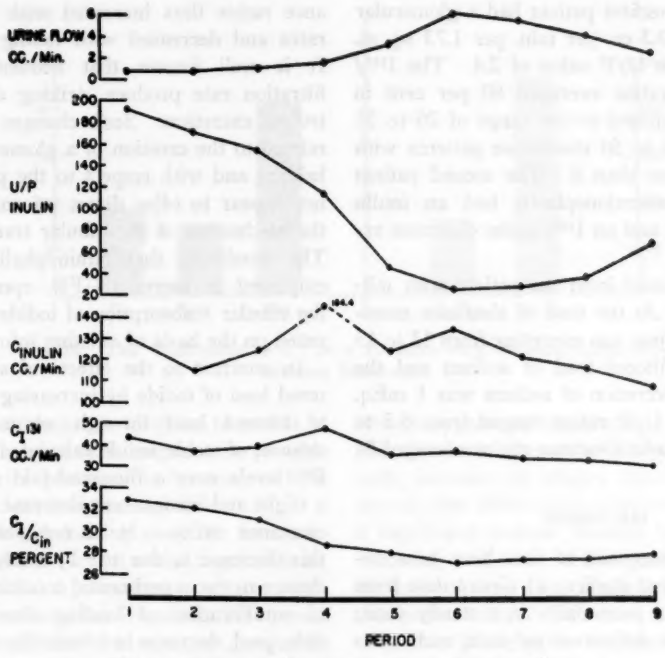

FIG. 9. EFFECTS OF INCREASING URINE FLOW ON C_{IN}

A. *Mannitol diuresis:* Two patients were studied during intravenous mannitol loading. The first case is shown in Table IV. Glomerular filtration rate remained relatively stable throughout the period of study. Inulin U/P ratios decreased from 20 to a minimum of 5. I^{131}/inulin clearance ratios rose from 31 per cent to 60 per cent and urea/inulin clearance ratios rose from 47 per cent to 77 per cent. The percentage of filtered water excreted increased from 5.7 to 22.

In the second patient subjected to mannitol diuresis the results were comparable to the above study. Inulin U/P values decreased from a maximum control value of 40 to a minimum of 7.8 and I^{131}/inulin clearance ratios increased from 32 to 53 per cent. The percentage of filtered water excreted increased from 2.7 to 14.

B. *Osmotic diuresis associated with renal disease:* Two patients with chronic glomerulonephritis, both with inulin U/P ratios below eight

TABLE IV

Osmotic diuresis after mannitol loading

Period	Time *min.*	V* *cc./min.*	U/P†	C_{in}‡ *cc./min.*	C_I/C_{in} %	C_{urea}/C_{in} %	V/wC_{in}§ %
1	0–16	5.3	18.8	99	31	47	5.8
2	16–29	5.2	17.8	93	33	47	6.1
3	29–41	4.4	21.1	93	32	46	5.2
Mannitol							
4	41–57	4.7	19.6	92	35	49	5.6
5	57–71	8.0	10.1	81	47	67	10.8
6	71–81	12.7	6.5	83	54	69	16.6
7	81–91	16.7	4.9	82	60	77	22.0

* V = urine flow.
† U/P = urine/plasma inulin ratio.
‡ Values corrected to 1.73 sq. m. body surface area.
§ Percentage of filtered water excreted was computed from the conventional formula V/wC_{in}, where V is the true urine flow, C_{in} is the filtration rate, and w is the correction for water content of plasma.

were studied. The first patient had a glomerular filtration rate of 9.3 cc. per min. per 1.73 sq. m. and a mean inulin U/P value of 2.4. The I^{131}/inulin clearance ratios averaged 80 per cent in this subject as opposed to the range of 26 to 51 per cent observed in 50 studies on patients with U/P ratios greater than 8. The second patient with chronic glomerulonephritis had an inulin U/P value of 4.4 and an I^{131}/inulin clearance ratio of 58 per cent.

Data were obtained from one patient with salt-losing nephritis. At the time of clearance measurements this subject was excreting from 13 to 15 per cent of his filtered load of sodium and the average rate of excretion of sodium was 1 mEq. per min. Inulin U/P ratios ranged from 6.5 to 8.7 and the I^{131}/inulin clearance ratios averaged 61 per cent.

DISCUSSION

Two general categories of data have been obtained in the present studies: a) *Group data* from individual subjects presumably in a steady state; and b) *Data from individual subjects,* each serving as his own control, in whom experimental conditions were acutely altered. The group data supply general information which is representative of the population. The individual studies allow for an examination of certain of the dynamic aspects of iodide excretion.

In the group data, a linear relationship between $C_{I^{131}}$ and C_{In} is apparent over a range of filtration rates from 40 to 155 cc. per min. Moreover, the values for $C_{I^{131}}$ were invariably less than the concurrent values for C_{In}. These observations imply that: a) $C_{I^{131}}$ is filtration dependent; and b) based on the assumption that plasma iodide is largely or completely filtered at the glomerulus, some of the filtered iodide ion is reabsorbed from the tubular urine. The nature of the tubular transport mechanism is not apparent in the group data and the individual studies include a series of experiments designed to elucidate the properties of this mechanism.

The filtration dependence of $C_{I^{131}}$ was examined in the presence of acute alterations of glomerular filtration rate. Acute increases or decreases in GFR were always accompanied by changes in $C_{I^{131}}$ which were in the same direction, but proportionately greater than those of C_{In}. I^{131}/inulin clearance ratios thus increased with rising filtration rates and decreased with falling filtration rates. It is well known that sudden alterations in filtration rate produce striking changes in electrolyte excretion. Such changes are presumably related to the creation of a glomerulo-tubular imbalance and with respect to the present study do not appear to offer direct information regarding the mechanism of the tubular transport of iodide. The possibility that Aminophylline, which was employed to increase GFR, specifically blocked the tubular reabsorption of iodide cannot be evaluated on the basis of existing information.

In contrast to the effects of elevating the filtered load of iodide by increasing GFR, elevation of filtered load through administration of an amount of stable iodide calculated to raise plasma I^{127} levels over a thousand-fold resulted in only a slight and inconsistent decrease in iodide/inulin clearance ratios. It is not yet clear whether this decrease is due to: 1) analytic error; 2) a change in the experimental conditions (*e.g.,* failure of equilibration of loading doses with the iodide, pool, decrease in filterability of iodide, *etc.*); or 3) a minor alteration in the transport of iodide relative to the filtered load. In any event, the data indicate that with large increases in plasma iodide concentrations, the rate of urinary excretion of inorganic iodide remains essentially proportional to plasma iodide levels. Reabsorption, therefore, must also remain proportional to plasma iodide levels. The relative independence between $C_{I^{131}}$ and plasma iodide levels, which has previously been noted by Childs, Keating, Rall, Williams, and Power (*2*), is more suggestive of passive reabsorption of iodide as a consequence of a concentration gradient between tubular urine and plasma than it is of active reabsorption. Thus, the clearance of a substance excreted by physical processes alone is essentially independent of plasma levels, whereas the clearance of a substance transported by an active reabsorptive mechanism is influenced in a characteristic manner by elevation of plasma levels beyond a critical point. It is quite possible that the present loading experiments did not elevate plasma iodide levels sufficiently high to demonstrate an iodide Tm, for despite a thousand-fold increase in plasma iodide concentrations, the latter were still present in micro quantities. If a transport maximum for iodide does exist, it

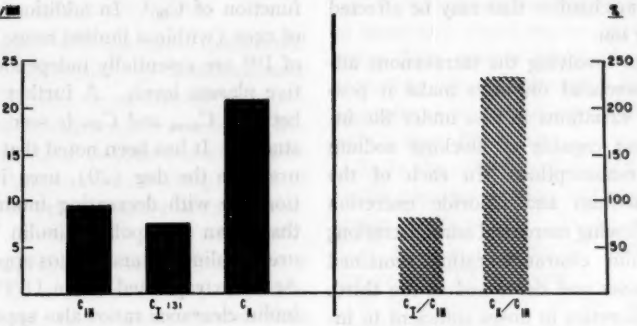

FIG. 10. THE CLEARANCE OF I^{131} IN A PATIENT WITH DEMONSTRABLE POTASSIUM SECRETION

must be geared to an iodide level far in excess of the physiologic range and it may not be possible to demonstrate its presence in humans without administration of toxic doses of iodide.

The data on iodide loading may also be examined from the point of view of evaluating iodide secretion. In studies of potassium excretion, loading with potassium salts has frequently been found to induce demonstrable secretion of potassium (13). Moreover, chronic loading with potassium facilitates the excretion of sudden large loads at more rapid rates and frequently elevates the clearance of potassium above that of inulin (13). In the present studies not only did C_I/C_{In} values not rise above unity after acute loading, but clearance ratios appeared to decrease slightly. In addition, chronic loading in two cases did not facilitate the excretion of sudden acute loads. In a subject with chronic renal disease, the opportunity to study iodide clearance in the presence of demonstrable K secretion was afforded (Figure 10). The potassium/inulin clearance ratio was 229 per cent, whereas the iodide/inulin clearance ratio was 79 per cent. Acute iodide loading in this patient resulted in a decrease in I^{131}/inulin clearance ratio to 70 per cent, rather than in an increase to a level exceeding unity. Thus, neither in this patient nor in any other subject studied to date has the value for $C_{I^{131}}$ exceeded that for C_{In}.

Thiocyanate ion has long been known to block the ability of the thyroid gland to trap inorganic iodide. Recently, it has been shown that thiocyanate also exhibits a rapid and definite blocking action on the iodide concentrating processes of the salivary gland (14, 15), although chloride secretion is apparently unaffected. Gastric secretion has also been altered by thiocyanate (16). Finally, increases in urinary chloride have been observed after thiocyanate administration (17), and a significant sodium diuresis has been reported following oral doses of thiocyanate ion (18). On the basis of these facts, it might be anticipated that thiocyanate should influence the renal excretion of iodide if an active mechanism is responsible for the tubular transport of this ion.

That the plasma concentrations of thiocyanate ion in the present patients were biologically active is suggested by the following: 1) In one subject salivary/plasma iodide ratios were measured before and after thiocyanate administration. The values for this ratio decreased 63 per cent and 53 per cent from control levels during the first and third clearance periods, respectively, after the administration of thiocyanate. 2) Plasma concentrations of I^{131} increased sharply in the two hyperthyroid patients and moderately in one of the two euthyroid subjects after NaSCN administration. These changes occurred during the infusion of I^{131} at a constant rate and probably resulted from a decrease in the volume of distribution of I^{131} due to a blocking of the thyroidal iodide trapping mechanism. Consonant with this explanation is the fact that plasma I^{131} concentrations failed to rise after thiocyanate administration to the myxedematous patient. 3) Sodium and chloride excretion rates increased after thiocyanate administration in four of the five cases.

The failure to demonstrate a significant thiocyanate effect on the I^{131}/inulin clearance ratios suggests that iodide is not handled by an active

tubular transport mechanism that may be affected by the thiocyanate ion.

The experiments involving the intravenous administration of mercurial diuretics make it possible to study the variations in $C_{I^{131}}$ under the influence of an agent capable of blocking sodium and/or chloride reabsorption. In each of the cases reported, sodium and chloride excretion rates increased following mercurial administration; however, I^{131}/inulin clearance ratios remained constant in two cases and decreased in the third. Thus, mercurial diuretics in doses sufficient to invoke moderate natriuresis and chloruresis did not noticeably decrease the tubular reabsorption of iodide.

Comparison of the clearance of I^{131} to the clearance of other electrolytes suggests that the tubular transport of iodide is not identical to that of sodium, chloride, or potassium, all of which are governed by active transport mechanisms. Thus, 1) the clearance of I^{131} (in cc. per min) is greater than C_{Na}, C_{Cl}, and C_K; 2) $C_{I^{131}}$ bears only an incidental relationship to the concurrent values for the clearances of sodium, chloride, and potassium in group data; and, 3) in individual studies altering the excretion rates of sodium, chloride, and potassium acutely within a limited range by administration of PAH, NaSCN, and mercurial diuretics, and by peritoneal puncture has not induced proportional changes in $C_{I^{131}}$.

The studies relating $C_{I^{131}}$ to rates of urine flow when the latter are decreasing are equivocal, but suggest at most a very slight relationship. $C_{I^{131}}$ does not appear to be influenced by rising rates of urine flow.

The osmotic diuresis data indicate that the clearance of I^{131} approaches the glomerular filtration rate as the inulin U/P ratio approaches one. This relationship shown in Figure 8 suggests that the tubular reabsorption of iodide may be a function of the proximal tubular reabsorption of water.

Certain points of similarity appear between the clearance of I^{131} and that of urea, a substance thought by most observers to be reabsorbed by passive back-diffusion. The data of Chasis and Smith (19) show that above a C_{In} of 40, C_{urea} is approximately a linear function of GFR. Above this level of C_{In}, I^{131} clearance is also a linear function of C_{In}.[7] In addition, both the clearance of urea (within a limited range) and the clearance of I^{131} are essentially independent of their respective plasma levels. A further point of similarity between C_{urea} and $C_{I^{131}}$ is seen in osmotic diuresis studies. It has been noted that during osmotic diuresis in the dog (20), urea/inulin clearance ratios rise with decreasing inulin U/P ratios such that at an extrapolated inulin U/P value of one, urea/inulin clearance ratios approach 100 per cent. At an extrapolated inulin U/P ratio of one, I_{131}/inulin clearance ratios also approach 100 per cent.

The present studies bring to light at least one possible difference between the excretion patterns for iodide and that for urea. The clearance of urea is influenced in a definite and predictable manner by changing rates of urine flow. The influence of decreasing rates of urine flow on $C_{I^{131}}$ as has been mentioned, is equivocal. If any relationship exists, it is not of the same order of magnitude as that noted for urea in the studies of Shannon in dogs (20) or Chasis and Smith in humans (19). In the presence of rising urine flows, it is well established that urea/inulin clearance ratios demonstrate a spurious rise (*i.e.*, "exaltation"). The present data fail to reveal a similar rise for I^{131}/inulin clearance ratios. One possible explanation for the poor relationship between $C_{I^{131}}$ and urine flow is that the distal tubule and collecting ducts may be relatively impermeable to the back-diffusion of iodide.

The majority of the present data appear most readily explicable on the basis of passive back-diffusion of iodide from tubular urine to plasma as a function of a concentration gradient created by the reabsorption of water from the glomerular filtrate. The fact that the iodide ion, despite its presence in trace quantities, must still be viewed as a charged particle does not appear to preclude diffusion as the mechanism of reabsorption.

[7] The linear correlation coefficient for the data of Chasis and Smith including all points where C_{In} exceeds 40 is 0.71 ($p = < .01$). The linear correlation coefficient for the present data is 0.82 ($p = < .01$). It must be noted that the urea data were obtained from subjects with glomerulonephritis, whereas the I^{131} data were obtained predominantly from patients without renal disease. This fact does not appear to invalidate the comparison.

SUMMARY AND CONCLUSIONS

1. The clearance of I^{131} has been determined (by constant infusion technique) simultaneously with other renal functions in an attempt to establish the mechanism of iodide excretion in the human kidney. Fifty-eight clearance studies were performed on 49 subjects with varying states of thyroid function and varying states of renal function.

2. In group data, $C_{I^{131}}$ has been noted to be a linear function of C_{In} over a wide range of filtration rates. Moreover, $C_{I^{131}}$ was always less than the concurrent value for C_{In}.

3. Acute increases and decreases in filtration rate were associated with changes of $C_{I^{131}}$ in the same direction as those of C_{In}, but these changes were always proportionately greater than those of GFR.

4. Increasing the filtered load of iodide by inorganic iodide loading resulted in slight decreases in I^{131}/inulin clearance ratios rather than; a) an increase in $C_{I^{131}}$ values asymptotically toward the concurrent values for C_{In} as might occur if a reabsorptive Tm had been surpassed, or b) an increase in I^{131}/inulin clearance ratios above unity as might occur if a tubular secretory mechanism existed.

5. The influence upon the renal clearance of I^{131} of agents capable of blocking renal tubular and/or iodide transport mechanisms has been investigated.

a) Sodium thiocyanate did not materially alter I^{131}/inulin clearance ratios, although plasma thiocyanate concentrations were adequate to alter plasma I^{131} levels and to change salivary/plasma I^{131} concentration ratios.

b) PAH loading was associated with slight increases in I^{131}/inulin clearance ratios in the presence of slight decreases in glomerular filtration rates. These alterations in the clearance ratios have been provisionally attributed to an osmotic diuretic effect of the loading agent.

c) Mercurial diuretics did not increase I^{131}/inulin clearance ratios despite a moderate natriuresis and chloruresis.

6. The clearance of I^{131} has been found to be essentially independent of the clearance of Na, Cl, and K in group data. In the presence of acute changes in excretion rates of Na, Cl, and K within limited ranges of the latter, $C_{I^{131}}$ exhibited only minor changes.

7. I^{131} clearance has been found to be influenced at most to a slight degree by decreasing rates of urine flow, over a range of inulin U/P values from 8 to 100 and apparently not at all by increasing rates of urine flow over a range of inulin U/P values from 190 to 11.

8. In the presence of osmotic diuresis, the I^{131}/inulin clearance ratios increased with decreasing inulin U/P values. At an extrapolated inulin U/P ratio of one, the I^{131}/inulin clearance ratio approached 100 per cent.

9. It is concluded on the basis of the present studies that iodide, in the human kidney, is filtered at the glomerulus and partially reabsorbed by the tubules. The data do not support the presence of an active tubular transport mechanism, and although this possibility cannot yet be excluded, it is provisionally held that iodide is reabsorbed predominantly by passive back-diffusion.

ACKNOWLEDGMENT

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PANTOTHENIC ACID DEFICIENCY INDUCED IN HUMAN SUBJECTS[1]

By WILLIAM B. BEAN, ROBERT E. HODGES, AND KATE DAUM WITH THE ADVICE AND TECHNICAL ASSISTANCE OF JAMES T. BRADBURY, RUTH GUNNING, JOSEFINE MANRESA, WILLIAM MURRAY, PATRICIA OLIVER, JOSEPH I. ROUTH, HAROLD P. SCHEDL, MARY TOWNSEND, AND I. CHU TUNG

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Bean and Hodges (1) gave a preliminary report of four young men in whom clinical disorders and metabolic faults were induced by a "diet" devoid of pantothenic acid, with omega-methylpantothenic acid, a vitamin antagonist (Figure 1). It is proposed here to describe details of the experiments, results, and some interpretations. The background for using molecular antagonists (2, 3) in human nutrition had been explored previously using antagonists to nicotinic acid (4).

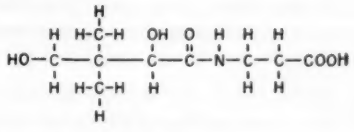
Among the accepted B-complex vitamins, pantothenic acid has been most puzzling in human nutrition. Its discovery by Williams, Lyman, Goodyear, Truesdail, and Holaday (5) and its presence in coenzyme A (6) suggested metabolic functions in man. Its employment in treating undernourished persons yielded no clue. Data from experimental animals revealed that the principal manifestations of its deficiency are those of adrenal cortical failure, accompanied by low plasma cholesterol, gastrointestinal symptoms and lesions, impaired antibody production, neurological lesions and alterations of the epidermis (7–11). The function of pantothenic acid in animals has been clarified by Drell, Dunn and their co-workers (12–15) who synthesized omega-methylpantothenic acid, demonstrated that it antagonized pantothenic acid, determined its toxicity in animals and showed that its deleterious effects could be reversed or prevented by pantothenic acid. Some of these observations were verified and extended by Shils (16).

PROCEDURES

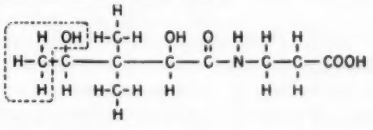
Early in 1950 we tried to induce pantothenic acid deficiency in a healthy young man. With the principle of metabolic antagonists well established, with pantothenic acid available for parenteral use, with several adrenal steroids and ACTH on hand, and with the clear understanding by the subject that he might suffer bad effects (17), we proceeded with an experiment of probable hazard. Several of us took small and then larger amounts of pantoyltaurine (18) for taste, irritating effects and any obvious sign or symptom. It produced no effect. After six months the first subject, eating a good diet, was taking 4 grams of pantoyltaurine a day without any observable effect on signs or symptoms. Since the hospital diet had more pantothenic acid than was reported in standard tables, a mixture of synthetic and purified preparations, supplemented with minerals and the known vitamins, except for pantothenic acid, was

CHEMICAL FORMULAE

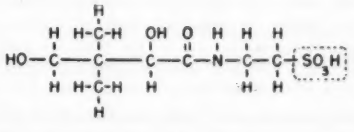
1. Pantothenic Acid



2. omega-Methylpantothenic Acid



3. Pantoyltaurine



4. β (γ-L-Glutamyl)-Aminopropionitrile

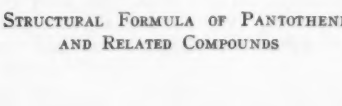


FIG. 1. STRUCTURAL FORMULA OF PANTOTHENIC ACID AND RELATED COMPOUNDS

[1] Supported by The Medical Department Trust Fund and grants from the Eli Lilly Company, The Nutrition Foundation and The National Vitamin Foundation.

TABLE I

Composition of formula		Vitamin supplement	mg.	Composition of salt mixture	mg.
Granulated sugar	290 gm.	Thiamine hydrochloride	1.2	Calcium biphosphate	67.9
Cornstarch	75 gm.	Riboflavin	1.8	Calcium lactate	163.5
Water	750 ml.	Pyridoxine	2.0	Ferric citrate	14.9
Vitamin-free casein*	125 gm.	Ascorbic acid	50.0	Magnesium sulfate	68.5
Corn oil	90 gm.	Niacin	12.0	Dibasic potassium phosphate	119.9
Cystine	750 mg.			Sodium biphosphate	43.6
Vitamin A	5330 U.S.P. units			Sodium chloride	21.8
Vitamin D	1070 U.S.P. units				
$NaHCO_3$	10 gm.				
Calories	3000				

Subjects

The three subjects† had the following characteristics:

M. C. was a 29-year old man who had always had excellent health. The past history was negative except for a fracture of the nose eight years previously and a Neisserian infection ten years previously. The height was 5 feet 9 inches and the weight was 137 pounds. He was normal except for a deviated septum and a few carious teeth. The arterial blood pressure was 110/60.

L. N. was a 32-year old man who had always had excellent health. His past history revealed pneumonia in childhood and dysentery in 1941 while in military service. His height was 5 feet 8 inches and his weight was 188 pounds. He was slightly fat and had some carious teeth. His arterial blood pressure was 130/80.

E. D. was a 27-year old man who had had excellent health. His past history revealed a few episodes of pleuritic pain in the left side of the chest a year previously and an attack of phlebitis in the leg many years ago. The physical examination revealed a normal man. X-ray of the chest revealed no lesion. His height was 5 feet 8 inches and his weight 138 pounds. His arterial blood pressure was 130/78.

* Contains 20 mcg. Vitamin B_{12} by assay.

† We were enabled to do the study with prisoners as volunteers through the thoughtful cooperation of Mr. Roy Purcell, warden of the Iowa State Reformatory at Anamosa, and with the authorization of the State Board of Control, Mr. Henry W. Burma, Chairman.

used. The formula (Table I) had to be given by stomach tube. Without any added antagonist it caused lowering of plasma cholesterol and some impairment of the eosinopenic response to ACTH. Clinical signs of deficiency did not appear.

To this pantothenic acid free diet we added the antagonist, pantoyltaurine (Figure 1). After twenty months elapsed with several periods of return to a normal diet, no obvious effect was produced by pantoyltaurine in quantities several hundred times greater than the average daily intake of pantothenic acid. The pantothenic acid free diet alone for four weeks or with pantoyltaurine for eight weeks led to no symptom and little metabolic change. We concluded that pantoyltaurine was not an antagonist or might even act as a vitamin. It was absorbed and excreted in the urine which inhibited growth of the test organism while pantoyltaurine was being given. Since no sign was produced, and no significant change occurred in any of the tests we concluded that the diet was adequate, and the antagonist inert in the dose used.

The next step was made possible when omega-methylpantothenic acid (Figure 1) became available (13–15). After we had tried it for immediate acute toxic or irritating effects we gave it to three volunteers. In the original subject it produced biochemical lesions, symptoms, and signs. The formula, vitamins and salt mixture were given in three equal parts three times a day by stomach tube (Table I). The subjects adjusted well to this method of getting nourishment. Water and black coffee were allowed as desired. The subjects were examined daily. They were not confined to bed but were under surveillance by a nurse or attendant at all times. The biochemical tests were done according to the plan seen in Figure 2 and Table II.

TABLE II

Schedule of tests and methods

Daily
- Acetylation of para-aminobenzoic acid (19)
- Urinary 17-ketosteroids (20)
- Urinary creatinine; 24-hour urine volume
- Body weight, basal and standing blood pressure and pulse

Twice a week
- Plasma cholesterol (21)
- Plasma cholesterol esters (21)
- Eosinophile response to ACTH (22, 23)

Once a week
- Gastric analysis
- Insulin sensitivity test (24–29)
- Glucose tolerance test
- Water diuresis test (30)
- Blood studies

Every two weeks
- Plasma protein tests (31, 32)
- Bromsulphalein test
- Color photographs

Irregular
- Ca, P, N, balances
- Excretion of thiamine, niacin and ascorbic acid (33–36)

CONDITIONS		I	II	III a	III b	III c
DURATION		12 Days	25 Days	6 Days	10 Days	14 Days
DIET		Special diet by stomach tube			Well balanced normal diet	Special diet by stomach tube
VITAMINS	Pantothenic Acid	30 mg/d	None	4000 mg/d	2000 mg/d	
	B-12	20 mcg	20 mcg	20 mcg	1000 mcg first 2 days	20 mcg
	Others	As in Table I	As in Table I	As in Table I	Twice quantity in Table I	
ANTAGONIST omega-Methylpantothenic Acid		None	500 mg/d		None	None
CORTISONE	E.D.	None	None	+	++	None
	M.C	None	None	None	+++	None
	L N.	None	None	None	+++	None

↓ A single intramuscular injection of 100 mg Cortisone on day 2, period III a

++ Subject E.D. 75 mg Cortisone orally day 1- Period III b
37.5 " " " " 2 " "
25 " " " " 3 " "
12.5 " " " " 4 " "

+++ Subjects M.C, L.N. 50 " " " " 1 " "
37.5 " " " " 2 " "
25 " " " " 3 " "
12.5 " " " " 4 " "

FIG. 2. THE PATTERN OF TEST PERIODS, DIET, ANTAGONISTS, VITAMINS AND OTHER THERAPY

The test ran from 23 March to 28 May, 1954.

Our original plan was to have three major experimental periods: I the control, II the deficiency, III the recovery, and, if time permitted, IV a repetition of the deficiency period. In actual fact the periods were arranged as follows: I, the control period, lasted for 12 days. Period II was the deficiency period. The only change was that the subjects got 0.5 grams of omega-methylpantothenic acid instead of pantothenic acid in the formula. This period continued for 25 days, by which time full-fledged manifestations of deficiency were apparent. Period III was divided into sections: III-a, 6 days, III-b, 10 days, and III-c, 14 days. The third period was to have been identical with the second except for 4 grams of pantothenic acid added to the diet every day. Early in this planned recovery period, though some improvement occurred, the well-being of the subjects was deteriorating. It was thought dangerous to continue. We gave cortisone as indicated in Figure 2 and a general diet during this 10-day period; the antagonist was omitted. Period III-c lasted 14 days. The synthetic diet was resumed, and pantothenic acid was given in the amount of two grams daily. No further antagonist was given. The men were observed at intervals for a few months after the experiment. They showed no signs of persistent disorder except subject E. D. He had slight edema in the leg in which he had had phlebitis a number of years before. There was no recurrence of neurological sign or symptom.

RESULTS

Clinical

Some of the pitfalls in nutritional experiments in man have been noted (37). In the subjects, general appearance, skin, and mucous membranes did not change. During Period II the subjects, hitherto cheerful, became quarrelsome, sullen, and petulant. At times they spent most of the day in bed. Somnolence was particularly evident in L. N. and E. D. From the 12th to 16th days of Period II each subject independently complained of numbness and tingling of the hands and feet. At first this was transient and occurred only during inactivity. Later paresthesias were a constant source of annoyance. The men frequently stamped their feet and shook or wrung their hands in an effort to restore normal sensation. E. D. had an especially disagreeable burning sensation of his feet. M. C. developed a steppage gait with weak dorsiflexion of the feet; the third complained of constant numbness of the hands. All subjects had hyperactive deep tendon reflexes, weakness of the extensor muscles and interossei of the hands, and inability to walk on tip-toe. Sensory perception remained intact for pain, thermal, vibratory and two-point sensation. Position sense was slightly impaired in one man. Plantar reflexes remained normal. The paresthesias cleared up completely in Period III-a but the motor changes did not. The motor changes cleared slowly in Period III-b and III-c.

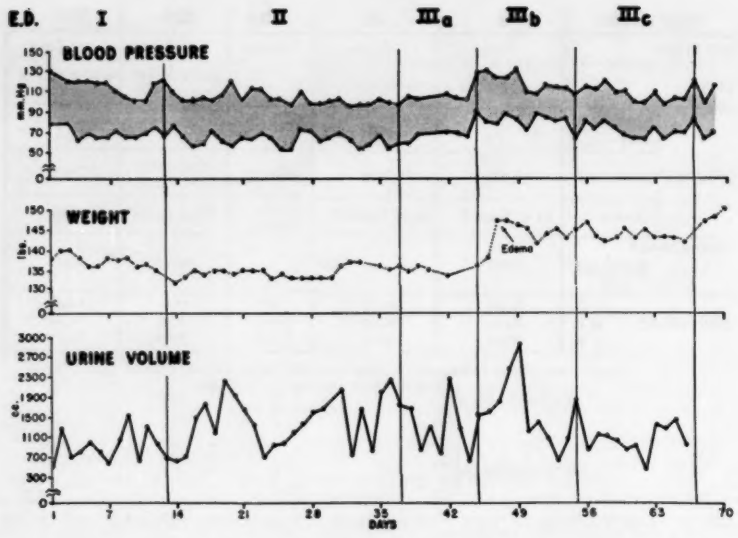


FIG. 3. REPRESENTATIVE CHART OF BLOOD PRESSURE, WEIGHT AND URINE VOLUME

Note the slight fall in resting blood pressure during Period II and the rise which coincided with the occurrence of edema. The edema persisted one to three weeks after cortisone was discontinued.

Gastrointestinal changes are the subject of a separate report (38).

In our first patient (J. S.) we noted lability of pulse rate and a very wide pulse pressure (1). The three subjects showed less evidence of cardiovascular changes (Figure 3), but during the deficiency period they had pulse rates of up to 120 following minor exertion. This was not accom-

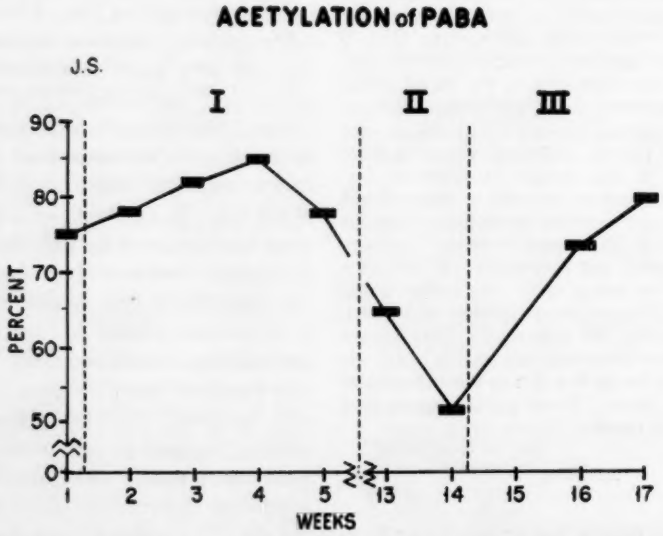


FIG. 4A. PER CENT OF EXCRETED PABA IN THE ACETYLATED FORM IN J. S.

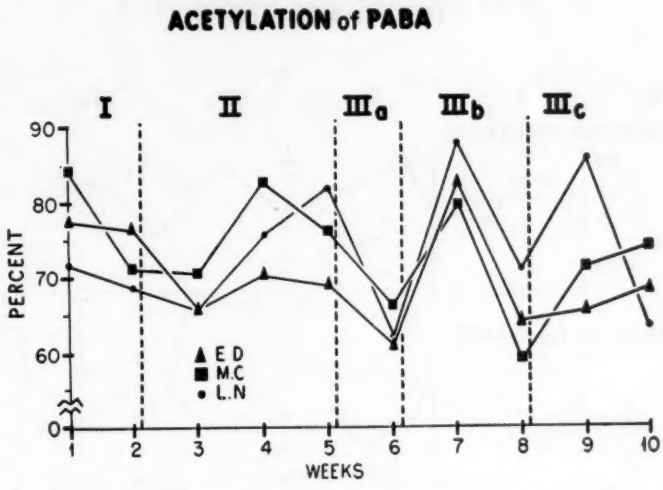


FIG. 4B. PER CENT OF EXCRETED PABA IN THE ACETYLATED FORM IN E. D., M. C., AND L. N.

panied by dyspnea or distress. The basal systolic and diastolic blood pressures decreased. They had some orthostatic instability, occasional dizzy spells, but no fainting. After replacement therapy and cortisone were started, all three subjects had a rise in blood pressure which then gradually returned to normal.

During the deficiency period the three men had almost continual upper respiratory infections, particularly acute pharyngitis. E. D. developed lobar pneumonia at the start of Period II. It responded rapidly to therapy with penicillin. His leukocyte count was 19,800 per cu. mm. with 91 per cent polymorphonuclears. Subsequent studies in two other subjects on the same diet, without the antagonist, given by tube for four weeks, revealed no infection. There was no infection in Period III-c.

The first subject had a steady loss of weight not reversed by replacement of pantothenic acid in the diet. This pattern did not occur in the other subjects. Their weight remained constant until Period III-b, when they suddenly gained about 10 pounds, developed 2 + ankle edema and a moderate rise in blood pressure. These events antedated cortisone administration in one subject but coincided with it in the other two. Edema disappeared and the weight returned to normal 8

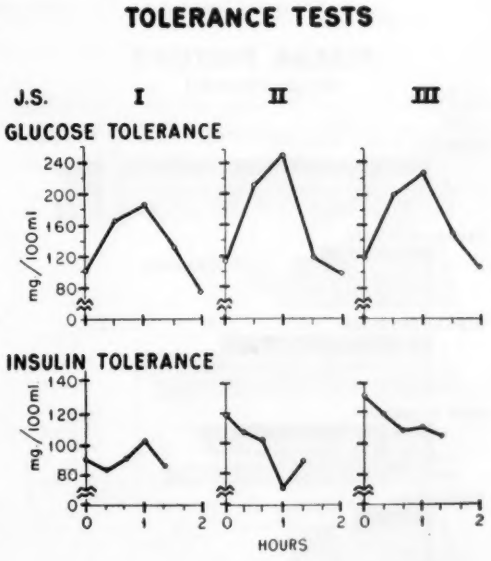


FIG. 5A. GLUCOSE TOLERANCE AND INSULIN SENSITIVITY CURVES IN J. S.

During the deficiency state the blood glucose rose to high levels, but did not remain elevated very long. At the same time insulin sensitivity increased. In Period II and subsequently 5 instead of 10 units of insulin were used.

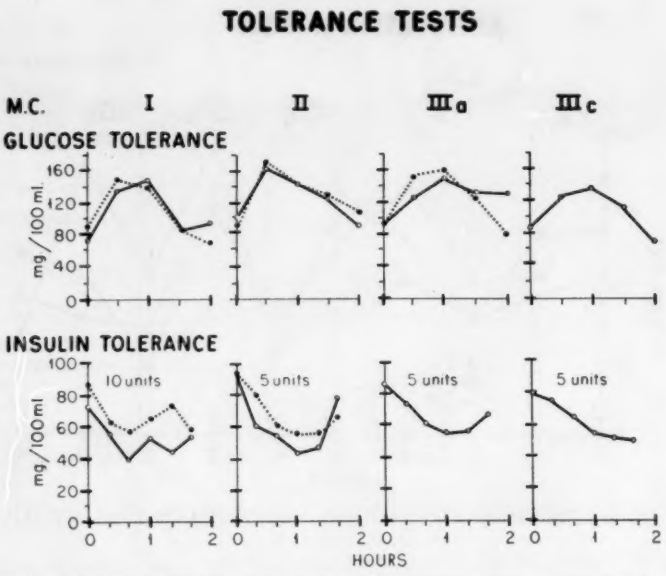


FIG. 5B. GLUCOSE TOLERANCE AND INSULIN SENSITIVITY TESTS IN SUBJECT M. C.
The dotted lines indicate the second test in a given period.

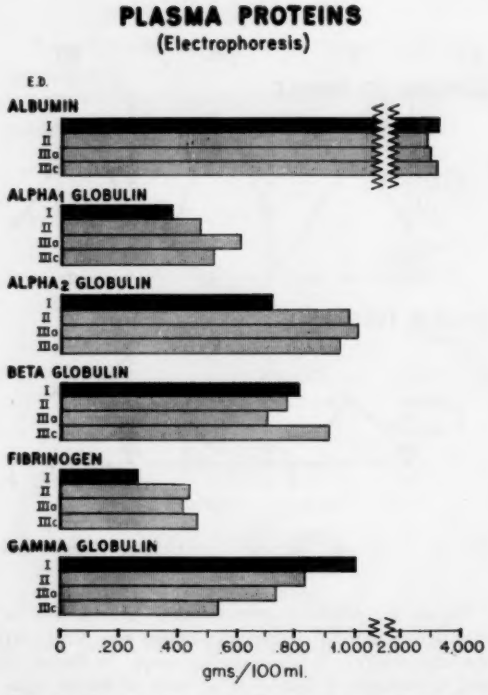


FIG. 6. PLASMA PROTEINS DETERMINED BY ELECTROPHORESIS

to 20 days after the administration of cortisone was stopped.

Metabolic studies

The subjects normally acetylated 70 to 80 per cent of the excreted PABA . In our first subject (Figure 4A) his percentage fell to about 50 per cent during the deficiency period. In the other three subjects (Figure 4B), however, the per cent of acetylated PABA remained near control levels until Periods III-b and III-c when it fell to 65 per cent. Administration of cortisone was followed by a prompt rise to 82 per cent.

In our first subject glucose tolerance tests showed an elevation in fasting and peak levels of blood sugar (Figure 5A). This pattern was not found in the other subjects (Figure 5B). Insulin tolerance tests, employing 10 units of regular insulin, resulted in troublesome hypoglycemia, so that during Period II, the dose was reduced to 5 units. All four patients had increased insulin sensitivity, which gradually disappeared during the recovery period.

Bromsulphalein tests were normal throughout. Urinary excretion of bile and urobilinogen was likewise normal. The normal serum albumin and

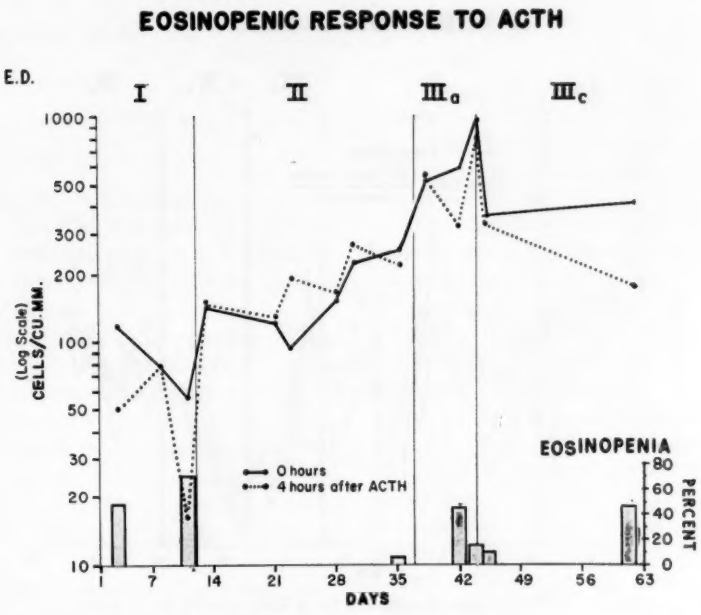


FIG. 7. EOSINOPHILE RESPONSE TO ACTH
Note the absolute rise of eosinophiles during Period II.

in most instances the normal glucose tolerance curves provided further evidence of normal liver function.

The albumin and total plasma proteins remained constant. However, the alpha$_1$- and alpha$_2$- globulins rose in two men and returned toward normal during the close of the observation period (Figure 6). Two of the three men had decreases in the gamma-globulin concentrations. E. D., with a progressive fall in this fraction, had continual severe upper respiratory infections.

During Period I, eosinopenic responses to ACTH usually were normal. In Period II the responses were distinctly impaired or absent (Figure 7). Following addition of pantothenic acid (Period III-a), the eosinopenic responses returned partially and were completely restored in Period III-c. The absolute eosinophile counts rose during the deficiency period and fell during the recovery period.

During Period I, there was great reduction in both cholesterol and esters, but the per cent of esters remained constant (Figure 8). During Period II, the cholesterol levels remained low but late in the period the esters fell as did the per cent of esters. When pantothenic acid was given (Period III-a) the cholesterol esters fell off sharply, but were restored quickly during the administra-

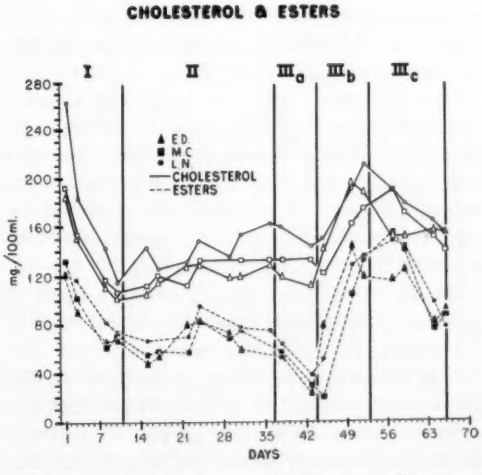


FIG. 8. CHOLESTEROL AND CHOLESTEROL ESTERS

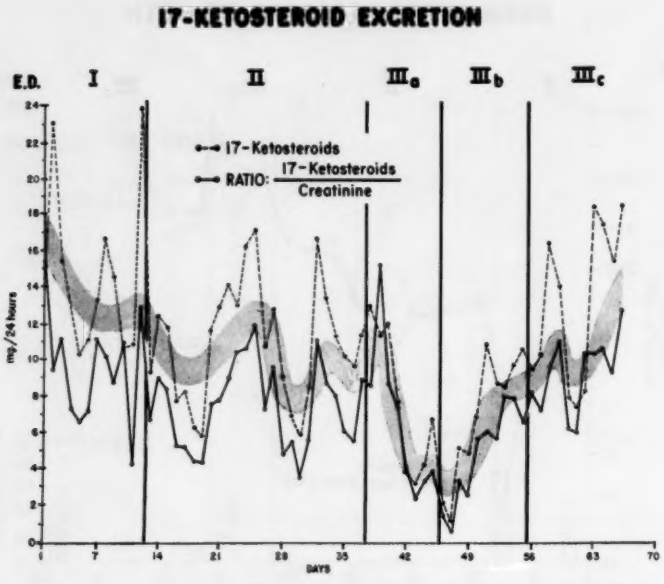


FIG. 9. 17-KETOSTEROID EXCRETION IN A REPRESENTATIVE SUBJECT

tion of cortisone, vitamins and a nutritious diet (Period III-b).

In subject J. S. there was a gradual decline in urinary 17-ketosteroid excretion during Period II. Similarly, in two of the three other subjects (Figure 9), the 17-ketosteroid excretion declined during the deficiency period. At the time pantothenic acid was restored to the diet, however, two subjects had a sudden fall in 17-ketosteroid excretion. The 17-ketosteroid excretion gradually rose during the recovery period (III-c).

During Period I the average plasma ascorbic acid levels were 1.6 and 1.5 mg. per 100 ml. and the average daily excretion in the urine was 40.3 and 74.0 mg. in subjects M. C. and L. N. At the end of two weeks of Period II, the plasma levels were zero. The daily urinary excretion had fallen to 19.1 and 38.7 mg., respectively.

During the control Period I in one subject 28 per cent of the dietary thiamine was excreted in the urine. This percentage rose to 58 per cent during Period II and fell to 19 per cent during the recovery period. For niacin, 41 per cent was excreted during the control period, 53 per cent during the deficiency period and 35 per cent during the recovery period.

Leukocyte counts were normal throughout. The only change in the differential leukocyte count was a rise in total eosinophiles during Period II. In one man they reached levels between 700 and 900 per cu. mm. During episodes of infection and fever, the expected leukocyte response occurred. The red cell counts and hemoglobin levels fell somewhat. A total of 540 ml. of blood was removed during the test (see Table III).

Routine urinalysis failed to demonstrate any abnormalities until the recovery period when L. N. developed a mild proteinuria which accompanied the elevation of blood pressure. Both urine and blood pressure became normal one week later. The somewhat irregular daily urinary output re-

TABLE III

Erythrocyte counts and hemoglobin

		E. D.	L. N.	M. C.
Initial	Hgb., *Gm./100 ml.*	15.3	12.7	14.7
	RBC in millions, *mm.*	34.56	4.32	4.39
End of Period II	Hgb.	13.0	11.5	13.7
	RBC in millions	4.18	3.98	4.54
End of Period III	Hgb.	11.2	11.0	12.6
	RBC in millions	4.04	4.18	4.36

mained adequate even when edema was developing in all three subjects.

DISCUSSION

Clinical impressions

Even without metabolic studies the severe clinical illness, the disturbance of neuromuscular function, and the profound physical and mental depression were clear testimony that the subjects' well-being was altered.

The notorious variability of reactions to, and descriptions of, subjective sensations (39–42) led to underrating the paresthesias. When tendon reflexes became hyperactive and steppage gait and foot drop occurred, the changes were obvious. The unpleasant sensations and later weakness may have come from a disorder of peripheral nerves but the muscles may have been the seat of the trouble.

We did not try to quantitate emotional, intellectual, or psychomotor functions. There was a deterioration in mood, the attitude now sullen and petulant, now quiet and somnolent. How much of the disorder was an emotional reaction to illness and how much was a specific effect of deficiency on the central nervous system is speculative.

The tendency to orthostatic instability varied erratically but did not parallel the complaint of weakness and clumsiness. Sometimes the subjects complained of dizziness, even at rest. There was no nystagmus and the Romberg test revealed unsteadiness no worse than with the eyes open. X-rays of the chest remained normal. The electrocardiogram revealed no change but sinus tachycardia. The abnormalities probably represent more than the neuro-circulatory adjustments to inactivity since they moderated during the recovery period when general activity was still curtailed by weakness.

The upward trend in blood pressure in Period III-b came after cortisone was given. Presumably the sudden effect of the steroid hormone may have been great because the adrenals were relatively inactive. This is in keeping with observations of Lewis and Page (43) on the reduction of induced hypertension in dogs by pantothenic acid deficiency. Nothing suggests an underlying renal disturbance; and the output of urine was very good during this as well as the other stages of the experiment.

The infections which were so troublesome cleared up after a good diet was given. They did not recur during Period III-c or in any subject without the antagonist so it is unlikely that irritation of the pharynx from the tube was a factor.

The relatively small changes in serum protein with an increase in $alpha_1$-globulin, $alpha_2$-globulin and fibrinogen and a decrease in gamma-globulin are in keeping with the possibility that specific antibodies were not being made adequately. Axelrod (44) and the Zuckers (45) have reported this in animals. There was no granulocytosis such as Weir has described in pantothenic acid deficient mice (46). The rise in eosinophile count was associated with no allergic state. The weight held steady until Period III-b when there was a sudden increase. In two this coincided with the administration of cortisone but in the third subject it began before the day before cortisone was begun.

There was a fall in acetylation during Period I continuing into Period II. Then there was a drift back toward normal and a consistent sharp fall in Period III-a when pantothenic acid was added to the deficient diet and the antagonist. There was a parallel decline in cholesterol esters (Figure 9), but the eosinopenic response to ACTH had come back. In Period III-b acetylation rose only to fall when the next tests were done (Figure 5). This rise may have been induced by cortisone. The fall occurred while clinical improvement was advancing. In the final phase (Period III-c) the trend was back toward normal except for subject L. N.

The sensitivity to insulin increased and we had to reduce the insulin dose from 10 to 5 units, and not give it intravenously. In what degree and indeed whether pantothenic acid deficiency produces this effect directly or through the mediation of the adrenal cortex we do not know. The liver functioned well.

The alterations of plasma protein fractions are similar to those discussed by Wintrobe, Jager, and Thorn (47) as reactions to stress and injury.

Changes in eosinophiles are consistent with the evidence that eosinopenia induced by ACTH depends on the functional integrity of the adrenal cortex, and that this power was lost during the induced deficiency state. Cognate evidence of

adrenal involvement and its reflection on eosinophiles is the consistent rise in absolute numbers of eosinophiles during the deficiency. They did not return to the baseline figures with the addition of pantothenic acid in Period III-a but came down later. ACTH, 25 mg. twice a week, may have disturbed the adrenal cortex.

The diet was devoid of cholesterol and had 90 grams of fat. The steady fall in cholesterol and its esters suggests that the levels of these compounds in the blood was reduced by their absence from the diet, but pantothenic acid metabolism may have been involved. During Period III-a when pantothenic acid was added cholesterol stayed the same but the esters dropped at the same time the 17-ketosteroid excretion was falling, eosinopenia induced by ACTH had returned, and acetylation was depressed. In Period III-c another roughly parallel decline in cholesterol and esters was not so fast nor so great as the initial fall. This difference may have been caused by the higher level of pantothenic supplementation, or by the residual effects of the cortisone and good diet.

The tendency for a diminished 17-ketosteroid excretion during Period II was exaggerated during Period III-a when pantothenic acid was given. This alteration occurred in phase with the fall in cholesterol esters, the decline in acetylation and the return of the eosinopenic response to ACTH. Excretion of 17-ketosteroids remained low until cortisone therapy was discontinued. It then rose steadily but slowly until the end of the experiment. We interpret these observations as indicating a disorder in adrenal cortical function induced by the deficiency in pantothenic acid.

Unpublished observations on two subjects were made by J. F. Manresa (48). In both subjects plasma ascorbic acid had become zero by the end of the second week of the deficiency period (ii), and the 24-hour excretion was reduced by half. No clinical signs of scurvy appeared. Beyond the well known connection of ascorbic acid and adrenal function (16) we have no idea what this observation signifies.

G. A. Amago (49) studied the excretion of thiamine and niacin in the urine of one subject. Excretion of thiamine rose significantly and excretion of niacin rose somewhat during pantothenic deficiency and returned to the control levels after the depletion period. One of the master puzzles in clinical nutrition concerns the interrelationships of the many individual vitamins of the B complex. Our observations are not adequate for general conclusions. It is unlikely that the increased vitamin excretion could have caused any of the observed clinical disorders. This needs to be tested by further investigation.

We tried to measure that adrenal cortical function determined by challenging the subject with a large drink of water (30). The data are unsatisfactory because by the end of the first week of the small feedings of Period I, drinking the large quantity of water caused prompt diarrhea by a gastrocolic reflex. As this was never wholly overcome the inadequate response to the water may have represented reduced absorption. The response was essentially normal in Period III-b. It was our impression that adrenal hypofunction was responsible for part of the low excretion.

Comments

We would emphasize particularly the small number of subjects and the considerable variations among them. The experiments were acute and severe. A milder deficiency lasting much longer might have produced a different state of disease. In the nature of our experimental plan we had to accept certain artifacts. Blood taken for tests probably contributed to the slight anemia. ACTH used to test eosinophile response no doubt had other effects on the adrenal cortex. The small volume of the formula and its low residue altered the bowel habits. Ingestion of a large amount of water was followed shortly by a watery diarrhea, thus obscuring any deterioration in the adrenal function of facilitating diuresis.

The production of physical illness, a neuromotor disorder and a series of biochemical disturbances by a diet devoid of pantothenic acid with an antagonist suggests that pantothenic acid is essential for man. We suggest further that the "burning feet" element of naturally occurring deficiency states may be mediated by pantothenic acid deficiency. This is in keeping with the clinical experiences reported by Gopalan (50). We are now testing a method of inhibiting some functions of the adrenal cortex by a reversible metabolic approach for a possible therapeutic role in ad-

vanced hypertension and diabetes and in various kinds of neoplastic disease. We are studying the possible connections of pantothenic acid metabolism with the dissecting aneurysm-kyphoscoliosis syndrome induced in rats by β-amino-propionitrile (Figure 1) (51, 52). This syndrome has some analogy with clinical lathyrism (42) and has some features in common with induced pantothenic deficiency. A diet with excessive quantities of sweet pea meal (Lathyrus odoratus) was the clue to finding the toxic factor producing the dissecting aneurysm-kyphoscoliosis syndrome in growing rats.

CONCLUSIONS

1. Subjects on a diet devoid of pantothenic acid, and with an antagonist, omega-methylpantothenic acid developed: 1) a neuromotor disorder; 2) cardiovascular instability especially in the upright position; 3) complaints referable to the alimentary canal; 4) repeated infections; and 5) physical and mental depression. They were not all relieved promptly by adding pantothenic acid to the diet.

2. The biochemical changes included alterations in acetylation, carbohydrate metabolism, blood cholesterol, steroid hormone excretion, plasma protein; and failure of ACTH to induce eosinopenia.

3. The individual variations were large.

ACKNOWLEDGMENTS

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THE EFFECT OF PANTOTHENIC ACID DEFICIENCY ON GASTRIC SECRETION AND MOTILITY

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Reports of animal experiments and results of preliminary studies by Bean and Hodges (1) in man indicated that the metabolic effects of pantothenic acid deficiency in human subjects would be widespread and profound. Such was the case (2). Insofar as it was possible to predict the metabolic changes in this experiment it seemed likely that one feature would be a suppression of adrenal cortical function and that this might lead to a secondary reduction of gastric secretion. On the other hand, following Lipmann's (3) demonstration that pantothenic acid is the active principle of coenzyme A, it has been shown that many metabolic processes involve acetylation reactions in which coenzyme A is essential (4). This led to the hypothesis that coenzyme A might play a fundamental part in the metabolism of parietal cells and that pantothenic acid deficiency would, therefore, have a direct inhibitory effect on their production of hydrochloric acid. These were the theoretical considerations which led to the special study of gastric function during pantothenic acid deficiency in the hope of obtaining information of physiological and possibly therapeutic interest. This paper reports the results of these studies which were undertaken concurrently with those reported by Bean, Hodges, and Daum (2).

MATERIALS AND METHODS

The subjects of the experiment were three normal young adult men who had no previous history of gastrointestinal disease. During the experiment there were five different dietary periods. Since a palatable diet deficient in pantothenic acid cannot be prepared from natural foods, a synthetic liquid formula was prepared and this was administered directly into the stomach through a plastic tube 5 mm. in diameter. This formula, which contained an adequate supply of all essential food factors, including pantothenic acid, was given for an initial control period of 12 days. For a second period of 25 days the same formula was used but pantothenic acid was omitted and omega-methylpantothenic acid, a metabolic antagonist to pantothenic acid, was substituted. In the third phase of the experiment both pantothenic acid and the antagonist were administered with the object of correcting the deficiency. After six days of this regimen the expected improvement in the manifestations of deficiency had not occurred and it became necessary to allow a general diet by mouth supplemented by pantothenic acid and other vitamins. At this stage there were manifestations of insufficiency of the adrenal cortex and cortisone was administered in small daily doses for four days (2). Three days after terminating cortisone therapy tube feeding was resumed and continued for nine days as a final control period. The original formula containing pantothenic acid, but not the antagonist, was administered during this final period.

Three tests were selected to compare gastric secretory and motor function during the development of deficiency with the normal function during the initial control period and during the final recovery period. These tests were a modification of the serial test meal devised by Hunt and Spurrell (5, 6), the secretory response to histamine, and the secretory response to insulin. For all these tests plastic tubes 5 mm. in diameter and having one terminal and four lateral holes were used. Saliva was collected throughout each test by an oral catheter attached to an electric suction pump.

Test meals were considered essential to the investigation because they provide a physiological stimulus to gastric secretion and supply information about the emptying pattern of the stomach. Neither basal secretion nor histamine nor insulin tests provide direct chemical and mechanical stimulation of the stomach mucosa and wall. However, since the concentration of hydrochloric acid in gastric contents is dependent both on the rate of secretion and the rate of emptying of the stomach, fractional methods are of little value where quantitative information about secretion is required (7). In the technique devised by Hunt a series of meals of constant volume and composition and containing phenol red are completely withdrawn after different intervals of time on different days. Complete emptying of the stomach at the beginning and end of each meal is ensured by lavage with a known volume of distilled water. From the results of a series of meals composite graphs of the secretion and emptying during the whole digestive phase can be constructed. Hunt has shown that viscosity is unimportant in a test meal and that the pectin in his original formula is unnecessary. He has found that a meal consisting of 750 ml. of sucrose solution containing 75 gm. sucrose and approximately 60 mgm. phenol red per liter

is satisfactory (8). It has been confirmed in this laboratory in eight normal subjects and patients with peptic ulcers that a meal of this composition will give remarkably constant results from day to day with regard to both secretion and motility. By estimating the concentration of phenol red in the original meal and in the stomach contents on each occasion it is possible to calculate the volume of the original meal remaining in the stomach. By assuming that the concentration of dye leaving the stomach through the pylorus during each interval is equal to the mean of the initial and final concentrations, the volume of gastric contents passing the pylorus during each time interval can be calculated. Penner, Hollander, and Saltzman (9) have shown that phenol red is suitable for this purpose as it is neither altered nor absorbed by the stomach. By a similar technique if the concentrations of hydrochloric acid, total chloride and pepsin in the gastric contents are estimated in each of the series of meals, the quantity of these substances which has passed the pylorus in between the time of withdrawal of each meal can be calculated. Since the volume of gastric contents at each time is known the amount of each constituent secreted at various stages of the digestive period can be estimated. In a series of 19 normal subjects Hunt found that the gastric secretory and emptying pattern of each individual remained remarkably constant from day to day. He also showed that if the volume of the original meal remaining in the stomach (calculated from the volume of gastric contents and the concentration of phenol red) is plotted against time for a series of meals an exponential curve is obtained.

In this laboratory exponential curves of gastric emptying have been obtained in 17 experiments on 15 normal subjects and patients with peptic ulcer, using a meal containing either pectin and sucrose 35 gm. per liter or sucrose 75 gm. per liter without pectin. The technique was, therefore, considered suitable for the demonstration of the effect of pantothenic acid deficiency on gastric motility. Any alteration of the emptying pattern during deficiency might be expected to alter the exponential curve obtained for each subject during the control period.

The results of the studies of gastric secretion in response to the test meals, to histamine and to insulin have been expressed as volumes of parietal and non-parietal component of gastric juice. These volumes may be calculated from the amount of hydrochloric acid and total chloride secreted during a given period by using a formula evolved by Hunt (10) from Hollander's (11) concept of two component secretion of gastric juice in dogs, and from Ihre's (12) figures for the composition of pure gastric juice in man. This method has the advantage of taking into account neutralization of hydrochloric acid by the alkaline component of gastric secretion.

In the present experiment the volume of gastric juice secreted changed to such a degree that construction of a composite graph for the volumes of parietal and non-parietal component was not possible. These volumes were therefore calculated for each individual meal from time zero to the time of withdrawal. Since gastric secretion is generally rapid and constant for the first 30 minutes after ingestion of the meal, this interval was selected for the comparison of test meal data obtained at different phases of the experiment. Where the actual time of withdrawal of the meal was close to 30 minutes this forms a sound method of comparison. Normal gastric secretion has been found to become slower after 30 minutes and there is no way in which meals of longer duration can be compared with those of 30 minutes when the values for each are calculated from time zero, instead of being incorporated in a serial graph. Meals of longer duration were included in the present experiment so that data on gastric emptying could be obtained, and so that any rise in secretory rate at a late stage in the digestive period would not be overlooked. The result of each of these meals was plotted on a graph of time against volume of gastric juice and a straight line was drawn from this point to time zero volume zero. The volume was read from the point at which this straight line crossed the line representing 30 minutes. It is realized that this method gives an approximate estimate only of the actual secretion at 30 minutes but since the figures obtained were not inconsistent with those obtained when the actual duration of the meal was 30 minutes, they have been included in the graphs of the results. This method would reflect any gross changes in secretion during the latter part of the digestive phase from one period of the experiment to another. Where the actual time of withdrawal was less than 30 minutes the same method of expressing the results was used. Since gastric secretion generally remains constant in rate for the first 30 minutes the method is probably more accurate in this instance. If some slowing of secretory rate did occur before 30 minutes this method would tend to produce unduly high readings for 30-minute secretion. The pepsin secretion was treated in the same way, graphs being drawn to determine the estimated total pepsin secretion at 30 minutes.

In order to determine the response of the parietal cells to direct chemical stimulation the volume of gastric juice secreted in response to the subcutaneous injection of histamine was measured during the initial control period, during the deficient diet and during the final control period. Wangensteen's apparatus was used to collect gastric secretion by continuous suction for 30 minutes before and 90 minutes after histamine. In addition, manual suction with a syringe was used at intervals of 30 minutes to check the patency of the tube. Blockage, when it occurred, was cleared by injecting small quantities of air. In view of the role pantothenic acid is believed to play in the metabolism of histamine (4) and the reduction of histaminase in the alimentary canal in adrenal insufficiency, (13) small doses (0.5 or 1.0 mgm.) of histamine phosphate were used. In addition each test was preceded by an injection of 2 ml. of the antihistamine, Thenfadil®, given intramuscularly, 30 minutes before the histamine dose to prevent untoward reactions. From the volume of gastric juice collected and the concentration of hydrochloric acid, total chloride and pepsin, the total amount of these constituents secreted in response to his-

tamine was calculated. Using the same formula as in the serial test meals, these figures were converted into volumes of parietal and non-parietal components of gastric juice.

The gastric response to insulin was included in the investigation since this provides an indication of the sensitivity of the receptor-conductor mechanism for neurogenic stimuli to gastric secretion. It may thus serve to differentiate factors acting directly on the parietal cells from those acting on the psychic or reflex secretion of gastric juice. Since pantothenic acid deficiency suppresses adrenal cortical function in animals it was expected that the subjects of the experiment would develop some hypersensitivity to insulin. Small doses (5 or 10 units) of insulin were, therefore, used and these were given subcutaneously instead of intravenously. This made it necessary to collect gastric juice for a period of 150 minutes after insulin instead of 60 or 90 minutes. The technique of collection of gastric juice was the same as in the histamine tests, the insulin injection being preceded by collection of basal secretion for 30 minutes. Finger-tip blood samples were collected for blood sugar estimation once before insulin and at intervals of 30 minutes thereafter. The lowest reading of blood sugar was usually obtained at 60 minutes and occasionally at 90 minutes after insulin. The average fall of blood sugar was 36 mgm. per 100 ml. and on only two occasions was the fall less than 30 mgm. per 100 ml. A level of 50 mgm. per 100 ml. in venous blood is generally considered a satisfactory hypoglycemia. As the concentration of sugar in capillary blood is approximately 10 mgm. per 100 ml. higher than in venous blood, and as the capillary blood sugar level fell to 60 mgm. per 100 ml. or less in every test in this experiment, the hypoglycemia induced by the method used was considered adequate. The volumes of parietal component and non-parietal component and the quantity of pepsin secreted in response to hypoglycemia were calculated in the same way as in the histamine tests and the test meals.

The hydrochloric acid concentration in all specimens was determined by electrometric titration against 0.1 N sodium hydroxide with pH 6.5 as the end-point (12), the sucrose solution used in the test meals having been adjusted to pH 6.5 before administration. Phenol red does not have a sufficiently sharp end-point for use as the titration indicator, but its presence in the test meal specimens obscures Topfer's reagent and phenolphthalein.

Total chloride was also estimated by electrometric titration against 0.5 N silver nitrate solution using a silver-silver chloride electrode (12). This method is convenient, accurate and not affected by the presence of phenol red in the specimens.

Pepsin concentrations were estimated by the method of Bucher, Grossman, and Ivy (14). The substrate was prepared from commercial dried bovine hemoglobin powder (15). Anson's (16) curve was used to convert the results to pepsin units.

RESULTS

These are expressed graphically as volumes of parietal and non-parietal component and quantity of pepsin secreted in response to equal stimuli at different stages of the experiment. Figures 1, 2, and 3 show the results obtained in each of the three subjects. The whole shaded area represents the total volume of gastric juice, the dark shaded area shows the volume of parietal component, and the light shaded area shows the volume of non-parietal component.

The first graph in each figure shows the secretory response to the test meals. Above the results of each test the time of withdrawal of the meal is shown. In all three subjects at least one meal in each control period is comparable in duration with a meal or meals during the second and third periods when the symptoms of deficiency were present. In many instances the duration of the test meals was sufficiently near to 30 minutes to justify direct comparison. This applies to meals 1, 6, 8, 9, and 10 in subject M. C. (Figure 1); to meals 1, 5, 6, 8, 9, and 10 in the subject L. N. (Figure 2); to meals 1, 5, 8, and 9 in subject E. D. (Figure 3). On the basis of these meals alone it can be seen that in the subjects M. C. and L. N. (Figures 1 and 2) there was a profound fall in the gastric secretion, both of parietal and non-parietal components, during the period when the pantothenic acid deficient diet was being administered, with recovery during the final control period. The estimated 30-minute volumes of secretion derived from the meals of less than 30 minutes are probably valid also, since these were all during the deficient period and the method tends to produce a value above the actual 30-minute secretion. The figures obtained from the meals of more than 30 minutes' duration are considered to show that no significant gastric secretory response occurred during the later stages of the digestive period. The subject E. D. (Figure 3) shows a slight but much less significant fall during the period on the deficient diet. None of the three subjects showed a significant fall in the secretion of pepsin in response to a test meal, though the response to histamine and insulin is severely affected.

In the second graph of Figures 1, 2, and 3 the secretory response to histamine is indicated. The

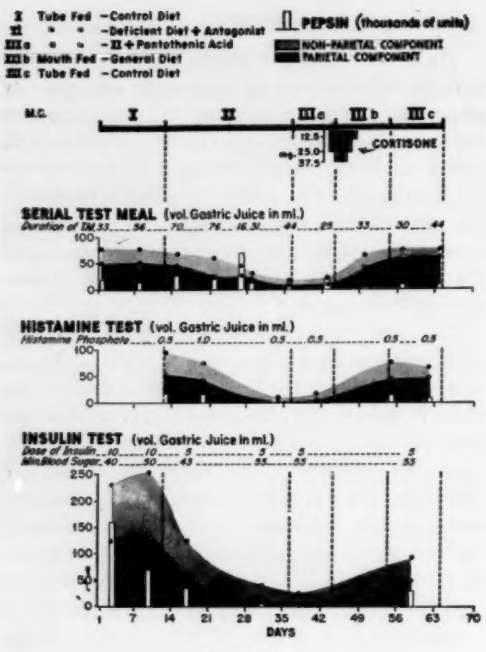
I Tube Fed – Control Diet
II " " – Deficient Diet + Antagonist
IIIa " " – II + Pantothenic Acid
IIIb Mouth Fed – General Diet
IIIc Tube Fed – Control Diet
PEPSIN (thousands of units)
NON-PARIETAL COMPONENT
PARIETAL COMPONENT
M.C.
CORTISONE
SERIAL TEST MEAL (vol. Gastric Juice in ml.)
HISTAMINE TEST (vol. Gastric Juice in ml.)
INSULIN TEST (vol. Gastric Juice in ml.)
DAYS

FIGURE 1

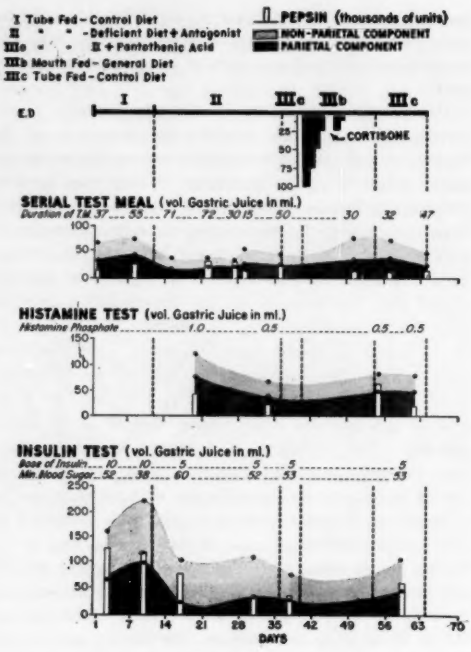
I Tube Fed – Control Diet
II " " – Deficient Diet + Antagonist
IIIa " " – II + Pantothenic Acid
IIIb Mouth Fed – General Diet
IIIc Tube Fed – Control Diet
PEPSIN (thousands of units)
NON-PARIETAL COMPONENT
PARIETAL COMPONENT
E.D.
CORTISONE
SERIAL TEST MEAL (vol. Gastric Juice in ml.)
HISTAMINE TEST (vol. Gastric Juice in ml.)
INSULIN TEST (vol. Gastric Juice in ml.)
DAYS

FIGURE 3

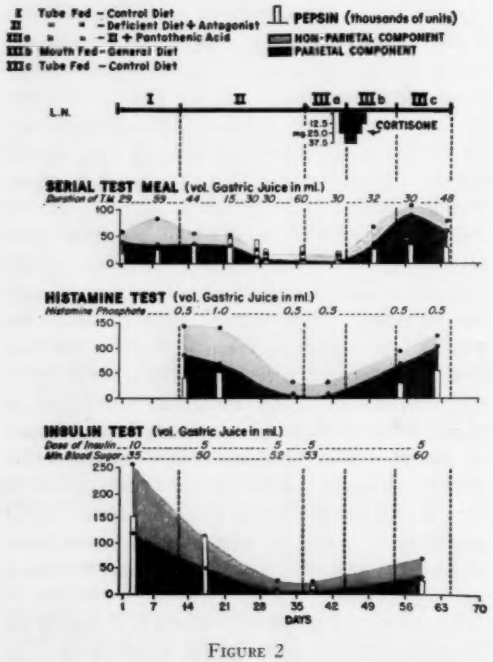
I Tube Fed – Control Diet
II " " – Deficient Diet + Antagonist
IIIa " " – II + Pantothenic Acid
IIIb Mouth Fed – General Diet
IIIc Tube Fed – Control Diet
PEPSIN (thousands of units)
NON-PARIETAL COMPONENT
PARIETAL COMPONENT
L.N.
CORTISONE
SERIAL TEST MEAL (vol. Gastric Juice in ml.)
HISTAMINE TEST (vol. Gastric Juice in ml.)
INSULIN TEST (vol. Gastric Juice in ml.)
DAYS

FIGURE 2

FIGS. 1, 2, AND 3. THE GASTRIC SECRETORY CHANGES DURING THE DEVELOPMENT OF PANTOTHENIC ACID DEFICIENCY IN THE SUBJECTS M. C., L. N., AND E. D.

The figures for the serial test meals show the estimated secretion at 30 minutes. Those for the histamine tests and insulin tests show the actual secretion during 90 and 150 minutes, respectively, after the test dose.

dose of histamine phosphate in mgm. is indicated above each test. Subjects M. C. (Figure 1) and L. N. (Figure 2) again show a striking fall in secretory response both of parietal and non-parietal component and of pepsin during the induction of pantothenic acid deficiency, with recovery during the final control period. The subject E. D. shows no significant change since the dose of histamine was greater in the first test than in the other three tests and there is no appreciable rise during recovery. The first histamine test in this subject was not obtained until seven days after beginning the deficient diet because of an attack of pharyngitis which made retention of a tube for 120 minutes impossible.

The third graph in each figure shows the secretory response to subcutaneous insulin. The dose of insulin and the lowest capillary blood sugar

reading obtained are indicated above the results of each test. As might be expected when there is a marked fall in response to histamine there is also a fall in response to hypoglycemia in subject M. C. (Figure 1) and L. N. (Figure 2). The subject E. D. (Figure 3) shows a definite though less marked fall in his gastric response to hypoglycemia.

The test meal results were used to determine the effect of pantothenic acid deficiency on gastric motility. The number of meals obtained during each phase of the experiment was not sufficient to prepare separate graphs for the initial and final control periods and for the period of the deficient diet. However, the logarithms of the volumes of the meals remaining in the stomach were plotted against time for each subject throughout the experiment. The results are shown in Figures 4, 5, and 6. In spite of gross changes in the volume of gastric juice secreted into the stomach the same exponential emptying pattern is retained by the subjects M. C. (Figure 4) and L. N. (Figure 5) throughout the experiment. The discrepant volumes found at 71 and 72 minutes in the subject E. D. (Figure 6) coincided with the pharyngitis already mentioned. His psychological reaction to the discomfort of being subjected to a test meal under these circumstances may explain the delay in gastric emptying (17). If they are excluded from the graph he also appears to have retained the same exponential emptying pattern.

DISCUSSION

The results of this experiment suggest that an adequate intake of pantothenic acid is essential to normal gastric secretory function, but not to normal motility. The conditions of the experiment were abnormal in that the three subjects were receiving a synthetic diet directly into the stomach instead of a natural diet by mouth. There is no report in the literature on the effect of prolonged tube feeding on gastric secretion but it is common experience that the use of a milk drip, in the treatment of peptic ulcer, causes a temporary neutralization of acid in the stomach rather than any suppression of secretion. If the results obtained were

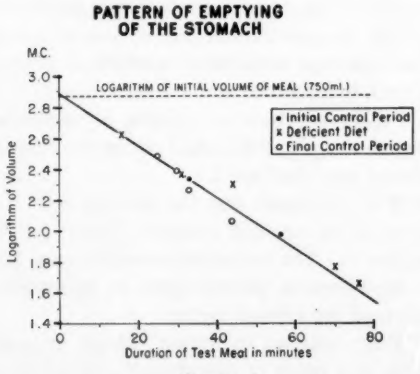

FIGURE 4

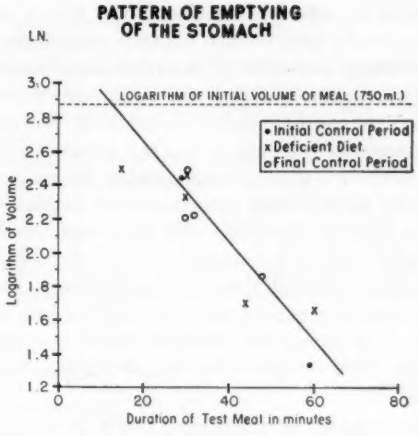

FIGURE 5

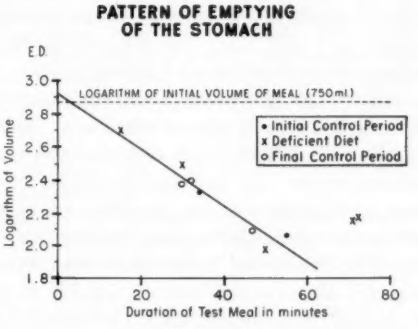

FIGURE 6

FIGS. 4, 5, AND 6. LOGARITHMS OF THE VOLUMES OF THE TEST MEAL REMAINING IN THE STOMACH AT VARIOUS TIME INTERVALS AFTER INGESTION PLOTTED AGAINST TIME OF WITHDRAWAL FOR THE SUBJECTS M. C., L. N., AND E. D.

Each graph includes results obtained during the initial and final control periods as well as during the period of pantothenic acid deficiency.

caused by tube feeding alone some fall of secretion should have become apparent during the initial control period of 12 days and improvement of gastric function during the final control period would be most unlikely. In addition, the pepsin secretion in response to the test meals would be expected to undergo suppression to an extent similar to the other constituents of gastric juice. It is believed, therefore, that pantothenic acid deficiency was in fact responsible for the secretory changes. Though there are many reports (4) of the effects of pantothenic acid deficiency on other tissue in animal experiments, there are no reports of the effects on the stomach of man or animals.

Pantothenic acid deficiency might bring about a depression of secretion of gastric juice by two possible mechanisms. Since coenzyme A is essential to the formation of cortisone by the adrenal cortex in rats (18) and since the subjects of the experiment showed evidence of adrenal cortical dysfunction, (1, 2), it is possible that the effects were due to a deficient supply of cortisone. Since it was necessary to administer cortisone and restore vitamins to the diet simultaneously, because of severe symptoms, it is not possible to say which factor was responsible for restoring normal secretion. The diminished hydrochloric acid secretion commonly found in Addison's disease is in keeping with such a mechanism. The absence of a fall in pepsin secretion in the test meals in the present experiment is inconsistent with the finding of Spiro, Reinfenstein, and Gray (19) that uropepsin is absent from the urine of patients with Addison's disease and could be restored by giving cortisone. There is, however, insufficient evidence to establish whether or not the diminished gastric secretion was mediated through the adrenal cortex.

Alternatively coenzyme A might be essential to the metabolic processes whereby the parietal cells secrete hydrochloric acid. The "electron-cycle" mechanism described by Crane, Davies, and Longmuir (20) postulates the enzyme substrate system oxaloacetate $\underset{-2H}{\overset{+2H}{\rightleftharpoons}}$ L-malate to account for the transfer of hydrogen atoms across the pericanalicular zone of the parietal cells. Coenzyme A presumably plays a part in this mechanism. Deficiency of pantothenic acid could theoretically block the mechanism of secretion at this point, if the electron-cycle mechanism is the true process by which hydrochloric acid is produced. Since the precise mechanism of hydrochloric acid secretion and the precise metabolic functions of pantothenic acid have not been established, any theory as to the relationship between the two must, at present, remain purely speculative. The results of the present experiment merely show that such a relationship does exist. Further work is now being undertaken with the object of investigating the means by which pantothenic acid deficiency inhibits gastric secretion.

The results of the experiment do not suggest a reason for the relatively minor effect on the gastric secretion in one of the three subjects (E. D.). In this particular subject the central nervous system manifestations of deficiency and evidence of insufficiency of the adrenal cortex were more severe than in the other two subjects. This may be a manifestation of the variations observed in many deficiency states where a group of individuals are all affected by the same deficiency. Individual subjects of the present experiment showed considerable variation in the degree and rate of change in the system or tissue predominantly involved (2).

SUMMARY AND CONCLUSIONS

1. Pantothenic acid deficiency was induced in a group of three normal human volunteers, by diet and the administration of a metabolic antagonist.

2. Two of three subjects developed severe depression of gastric secretion which returned to normal after the experiment had been terminated.

3. The changes affected the volume of parietal and non-parietal components secreted in response to a meal, to histamine and to insulin.

4. Pepsin secretion in response to insulin and histamine was also diminished but pepsin response to a meal was unaffected.

5. It is postulated that the parietal cells were depleted of an essential metabolic factor, possibly coenzyme A. The diminished secretion may, however, have been an indirect result of depression of function of the adrenal cortex.

6. There was no significant change in gastric motility as a result of pantothenic acid deficiency.

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THE EFFECTS OF SLEEP AND LACK OF SLEEP ON THE CEREBRAL CIRCULATION AND METABOLISM OF NORMAL YOUNG MEN [1]

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Numerous hypotheses have been elaborated in attempts to explain the puzzling phenomenon of sleep. Thus sleep has been attributed to arterial anoxemia, to cerebral ischemia or anoxia, or to a generalized narcosis on the basis of one or another metabolic alteration. Little information is available, however, on the subject of cerebral metabolism and function during natural sleep. This might be explained to a great extent by the difficulties inherent in any experimental investigation during so labile a state as physiological sleep. Moreover, reasonably quantitative techniques for measuring the blood flow and oxygen consumption of the brain in unanesthetized animals and in man have become available only recently. Early attempts (1) to obtain at least qualitative information on the cerebral blood flow in man during sleep by means of brain plethysmography through trephine holes have yielded contradictory results. Later attempts with better methods of recording (2) have suggested a decrease in cerebral blood flow on passage from the waking state to short or long periods of sleep. More recently, Gibbs, Gibbs, and Lennox have attempted to obtain a better understanding of the cerebral circulation during sleep by means of the thermoelectric flow recorder (3). They found no significant alteration in cerebral blood flow during short periods of sleep in four epileptic patients. Unfortunately, arterio-cerebral venous oxygen differences were not measured simultaneously, and no information on the important question of the oxygen consumption of the brain was obtained.

Recently developed methods permit a more quantitative determination of the cerebral blood flow and oxygen consumption during natural sleep. It was to obtain such information as well as to test some of the previously proposed hypotheses that the present study was undertaken.

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METHOD

Attempts to measure cerebral blood flow by means of the nitrous oxide technique (4) during sleep were made in approximately fifty, healthy, young, male volunteers varying in age from 17 to 36 years. In order to facilitate the induction of sleep under the conditions of the study, the subjects had remained awake for a period of approximately 20 hours, or about six hours beyond their normal bed-time, prior to the study. They are, therefore, referred to as "fatigued." Except for making the subjects as comfortable as the procedure would allow, no drugs or other special methods for inducing sleep were employed. The studies were performed in the early morning hours, most often between 4 and 6 A.M., when the tendency to fall asleep after a prolonged period of wakefulness is generally greatest (2). In most cases the subjects had been fasting for several hours; a few had eaten a light meal approximately two hours prior to the study.

In each study the subject was placed in the supine position, the needles were inserted in the internal jugular bulb and the femoral artery, needle electrodes for EEG recording were placed in the scalp, and a mask was strapped on the face. The subject was then permitted to rest in this position for about thirty minutes to permit any physical and emotional disturbances attending the introduction of the needles to subside. At this point the first or control blood flow determination was performed. The room was then darkened and quieted, and the subject, still in the same position, was given an opportunity to fall asleep. During this period the subject was usually permitted to breathe room air through a valve connected to the mask. In a few cases compressed air was fed from a tank into the mask through a reducing valve in an effort to minimize the waking effect of the gas flow associated with the nitrous oxide procedure. The state of wakefulness or sleep was followed by means of continuous EEG recordings in the adjacent room from the four to eight scalp electrodes previously inserted. These recordings were made continuously throughout the study

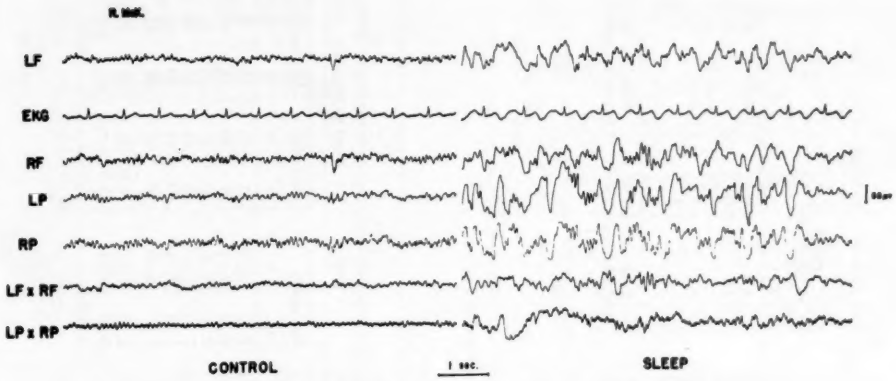

FIG. 1. ELECTROENCEPHALOGRAPHIC PATTERN DURING SLEEP

including the periods during which cerebral blood flow measurements were being made. The mental state or level of sleep was evaluated by means of the EEG record on the basis of the classification of Gibbs and Gibbs (5) as well as by clinical observation including movements, snoring, and response to whispered commands. In no case was the subject considered asleep until after the appearance of the characteristic sleep spindles and delta waves in the EEG record (Figure 1). When, on the basis of all these criteria, the subject was considered to be in a relatively steady state of sleep, a cerebral blood flow determination was made. The above procedure was followed in all the cases in Tables IA and IB except two (P. O. and McK.) in which the order was reversed, sleep occurring throughout the first determination of cerebral blood flow and the control determination performed after the subjects had been awakened and kept awake for 45 and 55 minutes, respectively.

In approximately fifty attempts an uninterrupted state of sleep of sufficient depth and duration to fulfill all the criteria was achieved in only six cases. A large number of studies was discarded because independent review of the EEG record failed to confirm the presence of a steady state of sleep throughout the period of cerebral blood flow determination or revealed momentary episodes of sleep rhythms during the control period. In some of those cases in which sleep did not occur, a second cerebral blood flow determination was made under exactly the same conditions as the first to form a group of "consecutive fatigued controls" with which to study the variation between two consecutive determinations in the same individual done under identical conditions but separated in time by an interval approximating that between the control and sleep determinations.

In all the studies mean arterial blood pressure was measured with a damped mercury manometer attached to the femoral arterial needle. Blood oxygen and carbon dioxide contents were determined by the manometric method of Van Slyke and Neill (6). Total hemoglobin concentration in arterial blood was measured in the Evelyn photometer according to a modification of the method of Evelyn and Malloy (7). Measurements of blood pH were made anerobically at room temperature by means of a glass electrode and Cambridge potentiometer and corrected to 37° C. by the factors of Rosenthal (8). Cerebral oxygen consumption, cerebral vascular resistance, and cerebral respiratory quotient were calculated as previously described (4). Blood carbon dioxide tension was computed by means of the nomograms of Peters and Van Slyke (9).

RESULTS

In Tables IA and IB are presented the data obtained in the six subjects on whom complete sleep studies could be made. The results obtained in the consecutive fatigued control studies in 13 subjects are presented in Tables IIA and IIB.

It is apparent that during sleep cerebral blood flow (CBF) is increased, rising from 59 during the control state to 65 cc. per 100 g. per min. during sleep ($p < 0.01$) while no such statistically significant change in CBF was found in the consecutive fatigued controls. The rise in CBF occurred in sleep despite a significant fall in mean arterial blood pressure (MABP) from 94 mm. Hg to 90 mm. Hg ($p < 0.05$). The series of consecutive studies on fatigued but awake subjects showed a significant blood pressure change in quite the opposite direction, rising from a mean of 90 mm. Hg in the first to 96 mm. Hg in the second determination ($p < 0.01$). This systematic change in MABP between two consecutive control determinations does not explain, however, the decrease found in sleep since the sleep series was a mixed one, sleep sometimes occurring in the first but more often

TABLE IA

Cerebral circulation and metabolism during sleep

Subject	Age	Interval *Min.*	MABP *mm. Hg* C*	MABP *mm. Hg* S†	CBF *cc./100 g./min.* C	CBF *cc./100 g./min.* S	$CMRO_2$ *cc. O_2/100 g./min.* C	$CMRO_2$ *cc. O_2/100 g./min.* S	$(A\text{-}V)O_2$ *Vol. %* C	$(A\text{-}V)O_2$ *Vol. %* S	CVR *mm. Hg / cc./100 g./min.* C	CVR *mm. Hg / cc./100 g./min.* S	Cerebral R.Q. C	Cerebral R.Q. S
E. R.	21	66	79	74	79	85	3.8	5.1	4.80	6.01	1.0	0.9	0.83	0.96
R. M.	28	30	107	95	45	48	2.6	2.3	5.70	4.75	2.3	2.0	0.94	0.86
S. W.	23	23	95	91	67	72	3.6	3.4	5.35	4.75	1.4	1.3	0.81	0.86
P. O.	23	45	99	96	54	61	3.5	3.1	6.51	5.12	1.8	1.7	0.93	1.02
H. K.	26	42	97	95	53	56	3.6	2.8	6.75	6.00	1.8	1.5	1.18	1.00
McK.	25	55	88	87	57	68	4.0	3.8	6.94	5.59	1.5	1.3	1.04	1.04
Mean	24.3	44	94.2	89.7	59.2	65.0	3.52	3.42	6.01	5.37	1.63	1.45	0.96	0.96
Stand. Error	±1.02	±6	±3.9	±3.4	±4.9	±5.3	±0.20	±0.39	±0.35	±0.24	±0.18	±0.15	±0.06	±0.03
p‡	—	—	<0.05		<0.01		>0.7		>0.1		<0.01		>0.9	

* C—Control—fatigued but awake.
† S—Sleep.
‡ Determined by method of paired comparison.

TABLE IB

Blood constituents during sleep

Subject	Hemoglobin concentration *Gm. %* C*	Hemoglobin concentration *Gm. %* S†	Blood O_2 content *Vol. %* Arterial C	Arterial S	Int. jugular C	Int. jugular S	Blood CO_2 content *Vol. %* Arterial C	Arterial S	Int. jugular C	Int. jugular S	Blood pH Arterial C	Arterial S	Int. jugular C	Int. jugular S	Blood CO_2 tension *mm. Hg* Arterial C	Arterial S	Int. jugular C	Int. jugular S
E. R.	14.43	14.55	18.90	19.58	14.10	13.57	47.06	46.83	51.04	52.62	7.31	7.29	7.24	7.27	47	50	60	58
R. M.	15.06	15.06	19.09	19.28	13.39	14.53	45.22	45.81	50.55	49.87	7.43	7.40	7.36	7.34	37	39	46	48
S. W.	14.25	14.43	19.81	19.97	14.46	15.22	50.31	51.28	54.65	55.38	7.33	7.34	7.30	7.30	49	49	56	57
P. O.	13.95	14.43	18.70	18.78	12.19	13.66	49.70	49.15	55.78	54.37	7.35	7.36	7.31	7.29	46	45	56	58
H. K.	14.94	14.86	20.98	20.58	14.23	14.58	52.14	54.63	60.13	60.62	7.35	7.35	7.31	7.31	49	51	60	61
McK.	13.16	12.81	19.01	18.38	12.07	12.79	51.67	51.28	58.86	57.10	7.35	7.38	7.33	7.36	48	44	56	51
Mean	14.30	14.36	19.42	19.43	13.41	14.06	49.35	49.83	55.17	54.99	7.35	7.35	7.31	7.31	46.0	46.3	55.7	55.5
Stand. Error	±0.28	±0.33	±0.35	±0.33	±0.43	±0.36	±1.10	±1.33	±1.60	±1.51	±0.02	±0.02	±0.02	±0.01	±1.9	±1.9	±2.1	±2.0
p‡	>0.6		>0.9		<0.1>0.05		>0.3		>0.7		>0.9		>0.7		>0.7		>0.8	

* C—Control—fatigued but awake.
† S—Sleep.
‡ Determined by method of paired comparisons.

TABLE IIA.—*Cerebral circulation and metabolism in consecutive control determinations in normal fatigued but awake subjects*

Subject	Age	Interval *Min.*	MABP *mm. Hg* I*	MABP *mm. Hg* II†	CBF *cc./100 g./min.* I	CBF *cc./100 g./min.* II	$CMRO_2$ *cc. O_2/100 g./min.* I	$CMRO_2$ *cc. O_2/100 g./min.* II	$(A\text{-}V)O_2$ *Vol. %* I	$(A\text{-}V)O_2$ *Vol. %* II	CVR *mm. Hg / cc./100 g./min.* I	CVR *mm. Hg / cc./100 g./min.* II	Cerebral R.Q. I	Cerebral R.Q. II
J. R.	27	110	86	97	63	66	3.0	2.9	4.70	4.37	1.4	1.5	0.74	1.10
A. A.	24	80	90	94	48	50	2.6	2.8	5.52	5.55	1.9	1.9	0.98	1.04
G. M.	22	70	82	86	54	53	3.0	3.3	5.63	6.28	1.5	1.6	0.98	0.94
R. O.	25	49	100	103	49	41	3.6	2.9	7.31	7.01	2.0	2.5	1.08	1.11
H. R.	24	120	81	102	52	48	4.0	3.3	7.62	6.86	1.6	2.1	0.95	0.99
J. D.	28	103	102	104	88	62	3.9	3.7	4.48	6.02	0.9	1.6	0.87	0.98
S. C.	19	125	87	89	56	68	3.7	3.4	6.57	4.77	1.6	1.3	0.86	1.15
T. F.	23	50	95	94	41	37	3.4	2.9	8.27	7.95	2.3	2.5	1.00	0.86
R. W.	22	83	88	100	77	74	3.8	3.8	4.96	5.19	1.1	1.4	1.00	1.08
W. O'D.	18	45	109	110	100	78	5.2	4.3	5.23	5.46	1.1	1.4	0.98	1.04
J. W. D.	29	120	80	86	61	57	2.8	4.2	4.66	7.47	1.3	1.5	0.81	0.94
P. T.	24	100	89	89	88	78	4.9	4.5	5.64	5.71	1.0	1.1	1.05	1.00
L. B.	22	90	83	94	66	68	3.6	3.8	5.45	5.65	1.3	1.4	0.82	0.98
Mean	23.6	88	90.2	96.0	64.8	60.0	3.65	3.52	5.85	6.02	1.46	1.68	0.93	1.02
Stand. Error	±0.9	±8	±2.5	±2.1	±5.3	±3.8	±0.21	±0.16	±0.34	±0.29	±0.12	±0 12	±0.03	±0.02
p‡	—	—	<0.01		>0.1		>0.4		>0.5		~0.01		<0.05	

* I—First control. † II—Second consecutive control. ‡ Determined by method of paired comparison.

TABLE IIB.—*Blood constituents in consecutive control determinations in normal fatigued but awake subjects*

Subject	Hemoglobin concentration *Gm. %* I*	Hemoglobin concentration *Gm. %* II†	Blood O_2 content *Vol. %* Arterial I	Arterial II	Int. jugular I	Int. jugular II	Blood CO_2 content *Vol. %* Arterial I	Arterial II	Int. jugular I	Int. jugular II	Blood pH Arterial I	Arterial II	Int. jugular I	Int. jugular II	Blood CO_2 tension *mm. Hg* Arterial I	Arterial II	Int. jugular I	Int. jugular II
J. R.	13.65	14.03	18.18	19.09	13.48	14.72	49.25	49.25	57.73	54.03	7.36	7.38	7.34	7.32	45	44	54	54
A. A.	13.48	13.39	18.56	18.57	13.04	13.02	49.78	49.42	55.17	55.20	7.46	7.47	7.41	7.36	37	37	45	50
G. M.	13.48	13.48	18.60	19.20	12.97	12.92	50.24	51.34	55.74	57.23	7.44	7.43	7.38	7.36	39	40	49	52
R. O.	15.08	15.29	20.89	20.88	13.58	13.87	44.22	46.04	52.05	53.81	7.37	7.37	7.34	7.34	40	42	50	51
H. R.	13.73	14.03	19.50	19.08	12.64	11.46	46.20	44.73	52.73	51.82	7.42	7.40	7.36	7.36	38	38	48	48
J. D.	14.75	14.66	20.23	20.49	15.75	14.47	49.06	47.71	52.94	53.59	7.40	7.40	7.35	7.36	42	41	49	49
S. C.	15.08	14.66	19.85	18.95	13.28	13.98	48.25	47.86	53.89	53.57	7.39	7.34	7.34	7.32	41	46	52	53
T. F.	16.14	16.27	22.31	22.13	14.04	14.18	47.28	46.78	55.56	53.62	7.40	7.39	7.36	7.33	42	42	53	53
R. W.	12.55	12.72	17.58	18.06	12.62	12.96	47.84	46.95	52.81	52.33	7.48	7.38	7.47	7.38	34	41	38	45
W. O'D.	13.10	13.27	17.89	18.28	12.66	12.82	49.22	48.80	54.35	54.56	7.42	7.41	7.38	7.40	40	40	47	46
J. W. D.	14.24	14.24	19.65	19.47	14.99	12.00	52.35	49.87	56.24	56.74	7.34	7.36	7.33	7.31	51	46	56	56
P. T.	13.10	13.48	18.18	18.31	12.54	12.60	49.25	49.57	55.17	55.27	7.43	7.44	7.36	7.33	39	39	50	54
L. B.	13.65	13.86	18.79	19.27	13.34	13.62	45.83	46.56	50.32	52.09	7.35	7.33	7.28	7.30	43	46	52	52
Mean	14.00	14.11	19.25	19.37	13.46	13.28	48.37	48.07	54.21	54.14	7.41	7.39	7.36	7.34	40.9	41.7	49.5	51.0
Stand. Error	±0.28	±0.26	±0.37	±0.32	±0.27	±0.27	±0.59	±0.51	±0.56	±0.46	±0.01	±0.01	±0.01	±0.01	±1.2	±0.8	±1.3	±0.9
p‡	>0.1		>0.3		>0.5		>0.3		>0.8		~0.2		>0.05		>0.3		<0.05	

* I—First control. † II—Second consecutive control. ‡ Determined by method of paired comparison.

TABLE III

Comparison between normal rested and normal fatigued young men

Subject	Age	MABP *mm. Hg*	CBF *cc./100 g./min.*	$CMRO_2$ *cc. O_2/100 g./min.*	$(A\text{-}V)O_2$ *Vol. %*	CVR *mm. Hg / cc./100 g./min.*	Cerebral R.Q.	Hb concentration *Gm. %*	Blood O_2 content *Vol. %* Arterial	Blood O_2 content *Vol. %* Int. jugular	Blood CO_2 content *Vol. %* Arterial	Blood CO_2 content *Vol. %* Int. jugular	Blood pH Arterial	Blood pH Int. jugular	Blood CO_2 tension *mm. Hg* Arterial	Blood CO_2 tension *mm. Hg* Int. jugular
Normal rested controls—11 cases																
W. C.	24	95	49	2.8	5.74	1.9	0.75	13.43	18.49	12.75	48.59	52.92	7.42	7.37	40	48
H. K.	20	88	42	2.2	5.25	2.1	0.84	13.85	19.61	14.36	48.66	53.09	7.44	7.39	38	46
R. C.	20	78	74	3.8	5.19	1.1	0.95	12.72	16.28	11.09	51.01	55.92	7.40	7.36	43	51
J. D. G.	22	91	80	3.8	4.77	1.1	1.08	13.94	19.29	14.52	49.20	54.36	7.41	7.35	41	51
E. L.	23	87	60	4.0	6.62	1.5	0.89	13.73	20.07	13.45	49.65	55.51	7.40	7.36	42	51
T. C.	24	88	49	2.9	5.97	1.8	0.84	12.18	17.12	11.15	46.55	51.58	7.45	7.39	35	44
L. H.	25	78	35	3.2	9.29	2.2	0.97	15.15	20.53	11.24	45.09	53.15	7.37	7.30	41	55
A. McC.	22	85	50	2.7	5.36	1.7	1.03	16.90	21.16	15.80	43.42	48.92	7.33	7.28	43	57
D. F.	19	85	54	4.3	7.89	1.6	0.92	15.08	19.56	11.67	43.90	51.19	7.34	7.28	42	56
L. H.	19	83	69	4.5	6.51	1.2	0.91	16.16	19.88	13.37	48.37	54.29	7.35	7.30	46	57
N. Y.	22	93	41	2.5	6.11	2.3	0.95	17.16	21.86	15.75	47.53	53.31	7.39	7.35	43	52
Mean	21.8	86.5	54.8	3.34	6.25	1.68	0.92	14.57	19.44	13.20	47.45	53.11	7.39	7.34	41.3	51.6
S.E.	±0.63	±1.7	±4.3	±0.23	±0.40	±0.13	±0.03	±0.50	±0.49	±0.53	±0.73	±0.60	±0.01	±0.01	±0.9	±1.3
Normal fatigued controls—25 cases																
M. S.	36	95	70	5.0	7.15	1.4	1.07	14.23	20.20	13.08	49.19	56.83	7.38	7.32	44	57
J. F.	25	107	56	4.2	7.45	1.9	0.91	13.48	19.38	11.93	51.23	58.02	7.39	7.37	44	53
T. I.	24	90	60	3.4	5.60	1.5	0.90	13.10	18.92	13.32	48.12	53.27	7.44	7.33	38	52
A. P. H.	17	89	70	3.2	4.56	1.3	0.82	12.35	18.13	13.57	50.06	53.99	7.40	7.38	42	47
S. D.	22	81	60	4.4	7.24	1.4	0.96	13.86	19.10	11.86	42.67	49.60	7.35	7.32	40	49
S. S. K.	34	98	57	3.7	6.45	1.7	0.96	13.65	18.86	12.41	44.96	51.15	7.31	7.27	45	56
J. Fr.	26	79	74	4.2	5.69	1.0	0.94	12.35	17.00	11.31	48.29	53.61	7.41	7.34	39	50
P. K.	22	83	62	3.4	5.59	1.3	0.95	13.89	18.80	13.21	52.28	57.57	—	—	—	—
H. K.	26	97	53	3.6	6.75	1.8	1.18	14.53	20.98	14.23	52.14	60.13	7.35	7.31	49	60
E. R.	21	79	79	3.8	4.80	1.0	0.83	14.03	18.90	14.10	47.06	51.04	7.31	7.24	47	60
S. W.	23	95	67	3.6	5.35	1.4	0.81	13.86	19.81	14.46	50.31	54.65	7.33	7.30	49	56
R. M.	28	107	45	2.6	5.70	2.3	0.94	15.09	19.09	13.39	45.22	50.55	7.43	7.36	37	46
J. R.	27	86	63	3.0	4.70	1.4	0.74	13.65	18.18	13.48	49.25	57.73	7.36	7.34	45	54
A. A.	24	90	48	2.6	5.52	1.9	0.98	13.48	18.56	13.04	49.78	55.17	7.46	7.41	37	45
G. M.	22	82	54	3.0	5.63	1.5	0.98	13.48	18.60	12.97	50.24	55.74	7.44	7.38	39	49
R. O.	25	100	49	3.6	7.31	2.0	1.08	15.08	20.89	13.58	44.22	52.05	7.37	7.34	40	50
H. R.	24	81	52	4.0	7.62	1.6	0.95	13.73	19.50	12.64	46.20	52.73	7.42	7.36	38	48
J. D.	28	102	88	3.9	4.48	0.9	0.87	14.75	20.23	15.75	49.06	52.94	7.40	7.35	42	49
S. C.	19	87	56	3.7	6.57	1.6	0.86	15.08	19.85	13.28	48.25	53.89	7.39	7.34	41	52
T. F.	23	95	41	3.4	8.27	2.3	1.00	16.14	22.31	14.04	47.28	55.56	7.40	7.36	42	53
R. W.	22	88	77	3.8	4.96	1.1	1.00	12.55	17.58	12.62	47.84	52.81	7.48	7.47	34	38
W. O'D.	18	109	100	5.2	5.23	1.1	0.98	13.10	17.89	12.66	49.22	54.35	7.42	7.38	40	47
J. W. D.	29	80	61	2.8	4.66	1.3	0.81	14.24	19.65	14.99	52.35	56.24	7.34	7.33	51	56
P. T.	24	89	88	4.9	5.64	1.0	1.05	13.10	18.18	12.54	49.25	55.17	7.43	7.36	39	50
L. B.	22	83	66	3.6	5.45	1.3	0.82	13.65	18.79	13.34	45.83	50.32	7.35	7.28	43	52
Mean	24.4	90.9	63.8	3.70	5.94	1.48	0.94	13.86	19.18	13.27	48.41	54.20	7.39	7.34	41.9	51.2
S.E.	±0.9	±1.9	±2.9	±0.14	±0.22	±0.08	±0.02	±0.18	±0.23	±0.19	±0.51	±0.54	±0.01	±0.01	±0.9	±1.0
p*	>0.05	>0.1	>0.05	>0.1	>0.3	>0.1	>0.6	>0.1	>0.5	>0.8	~0.3	>0.2	>0.9	>0.8	>0.6	>0.8

* Determined by method of significance of difference between two means.

in the second of the two determinations. It seems warranted to conclude that the decreased MABP is associated with the phenomenon of sleep. The elevation of the blood pressure in the second of the consecutive controls was probably the result of the growing discomfort on the part of the subject from lying in the same position for a prolonged period.

Since CBF increased despite a decreased MABP, cerebral vascular resistance (CVR) must have fallen in sleep, as indicated in the change in its calculated value from 1.6 in the control determinations to 1.5 mm. Hg per cc. per 100 g. per min. during sleep ($p < 0.01$). On the other hand, CVR rose from a mean of 1.5 in the first to 1.7 mm. Hg per cc. per 100 g. per min. in the second of the consecutive control determinations ($p \sim 0.01$). The fall in CVR observed in sleep is probably associated with the phenomenon of sleep and, for the same reasons discussed in relation to the MABP, cannot be explained simply by the systematic difference in CVR found to exist between two consecutive control determinations.

Cerebral arterial-venous oxygen difference and cerebral oxygen consumption (CMR_{O_2}) showed no significant changes in either the sleep or the consecutive control studies. Cerebral R. Q. did not change in sleep, but the mean value, 1.02, in the second of the consecutive control determinations significantly exceeded the mean value, 0.93, obtained in the first determination ($p < 0.05$), a finding for which no reasonable explanation is at hand. Arterial hemoglobin concentrations, arterial and cerebral venous oxygen and carbon dioxide contents and pH did not change significantly in either group. Arterial and internal jugular carbon dioxide tensions (pCO_2) were not significantly altered by sleep although it is interesting and possibly significant to note that these values were appreciably higher in the sleep group, even in their control state, than in the fatigued group which could not sleep during the studies ($p < 0.05$ and $p < 0.02$, respectively). Similarly, the mean values for arterial and internal jugular blood pH were significantly lower in both determinations of the sleep group than in the fatigued group which failed to sleep ($p < 0.05$, respectively). In the consecutive control studies arterial pCO_2 remained unchanged, but the mean value of cerebral venous pCO_2, 51 mm. Hg, in the second determination significantly exceeded the mean value, 50 mm. Hg, in the first determination ($p < 0.05$). This finding is probably related to the tendency for the CBF to decrease in the second determination.

In Table III, comparison is made between the results obtained in 25 awake but "fatigued" normal young men and those observed in 11 normal rested young men studied similarly by the same group of investigators. The values obtained in the rested subjects agree closely with those previously reported by Kety and Schmidt (4). These data are taken from the control values of various experimental procedures provided that the control determinations were made first. Thus the fatigued group includes also the first of the consecutive control determinations in Tables IIA and IIB and the control values of those sleep cases in Tables IA and IB in which the control determination was first. On the basis of this comparison, no significant differences between fatigued and rested subjects could be found although the mean value for CBF, 64, in the fatigued group exceeded the mean value, 55 cc. per 100 g. per min., in the rested group by an amount approaching statistical significance ($p < 0.1 > 0.05$).

DISCUSSION

These findings are of interest because of their pertinence to certain theories which have been advanced from time to time toward an explanation of the phenomenon of natural sleep. A number of these theories have in common the postulate that sleep is associated with and caused by a diminution in the gross nutrition or metabolism of the brain.

Quite recently, Doust and Schneider have elaborated a theory which ascribes sleep to arterial anoxemia and its resultant cerebral anoxia (10). On the basis of a downward drift in the readings of an ear oximeter, these authors concluded that arterial oxygen saturation progressively decreased during the process of falling asleep and reached levels as low as 87 per cent during deep sleep. The lack of an attempt to confirm this surprising result by more direct techniques and the absence of similar observations on non-sleeping controls leaves open the possibility, however, that it may have been one of the artifacts sometimes associated with indirect oximetry. Our findings (Table IB)

that both the oxygen content and hemoglobin concentration of arterial blood were normal during the control period and remained unaltered during sleep make unlikely any hypothesis which attributes a causal role to arterial anoxemia.

If not the first, then certainly one of the earliest recorded theories of sleep attributed this phenomenon to an ischemia of the brain. By recording changes in intracranial volume in two subjects with cranial defects, Mosso (1) concluded that sleep was associated with a decrease in cerebral blood volume. Tarchanoff (11) supported this view by observing a blanching of the pial vessels in puppies when sleep occurred. A large number of investigators, however, were unable to corroborate the findings of Mosso or found instead evidence of cerebral vascular engorgement (12–16). None of these observations yielded information on cerebral blood flow which is certainly different from and not necessarily correlated with cerebral blood volume. Only in the case of Gibbs, Gibbs, and Lennox, whose thermoelectric technique would probably have indicated if it did not measure gross changes, had observations related to cerebral blood flow in sleep been made (3). These authors were unable to demonstrate any change during sleep in the function which they studied.

The present studies show a moderate but statistically significant increase in cerebral blood flow associated with sleep. This occurred in the face of the slight but significant fall in arterial blood pressure seen in these subjects and consistently observed by numerous previous investigators (17–19) and was the result, therefore, of a decreased resistance to flow somewhere in the brain. Since the intracranial pressure is usually found to rise during sleep (14, 15), and since the present studies demonstrate no change in hemoglobin concentration in the blood, factors of decreased external pressure or lessened viscosity appear to be ruled out, and it seems warranted to conclude that there is a relaxation in cerebrovascular tone during sleep. This supports previous observations which suggested a cerebral hyperemia during sleep (12–16). The cause of this cerebrovascular relaxation remains obscure. It cannot be attributed to changes in arterial oxygen or carbon dioxide tension since these remained relatively unaltered between control and sleep, nor was it in response to an increase in cerebral metabolism which was also unaffected. The usually plausible hypothesis of a decrease in neurogenic vasoconstrictor tone is rendered less tenable by the failure to demonstrate a normal vasoconstrictor tone in the cerebral vessels of man, or at least one mediated by the known sympathetic inflow to the head (20).

Examination of the data in Table III reveals that the results in normal rested young men were almost identical with those in the original report of the method (4). When considered as a single group, the subjects who had remained awake for several hours beyond their normal bedtime and were, therefore, "fatigued," did not differ significantly in any respect from the rested young men. Within the "fatigued" group, however, arterial and cerebral venous carbon dioxide tensions were significantly higher and pH significantly lower in those subjects who slept than in the subjects who were unable to sleep under the conditions of the experiment (Tables IB and IIB). These differences, indicative of a mild respiratory acidosis, were apparent not only during sleep but even during the control period when the sleep subjects were awake. Comparable changes have been observed previously during sleep (2, 21), and Mills (22) has found elevations of alveolar carbon dioxide tension during the night or early morning irrespective of whether the subjects were awake or asleep. He attributes these changes to a normal diurnal rhythm in alveolar carbon dioxide tension which is independent of sleep. Our results indicate also that the respiratory acidosis can occur in the absence of sleep and that sleep *per se* causes little if any change in carbon dioxide tension and pH of the blood, but they raise an interesting question of whether sleep can occur in the absence of the respiratory acidosis. It was this finding which distinguished the subjects who slept from those who could not sleep under identical experimental conditions. Despite the evidence of respiratory depression, no significant anoxemia was observed in these subjects, nor was it to be expected. As Mills has also observed in his studies (22), the degree of carbon dioxide retention was insufficient to account for any appreciable fall in arterial-oxygen saturation, certainly not to the levels on which Doust and Schneider (10) based their anoxemic theory of sleep.

Another hypothesis on the nature of sleep suggests that this state is some endogenous narcosis associated with an overall decrease in metabolic activity in the central nervous system which permits the replenishment of certain substrate stores presumably depleted by the active metabolism of the waking state. That sleep is quite different from anesthesia or coma is clearly demonstrated by the data on cerebral oxygen consumption (Table IA). Whereas coma (23, 24) or anesthesia (25) are associated with profound decreases in the utilization of oxygen by the brain, this function in sleep is not significantly different from its level in the waking state.

Thus the state of sleep should be added to a growing list of conditions, like schizophrenia (23) and performance of mental arithmetic (26), in which a good correlation between energy conversion and functional activity commonly found in other organ systems appears to be absent. This result is compatible with the current vogue of viewing the brain as a calculating or communicating mechanism which, in contradistinction to machines which do mechanical work, utilizes by far the greater part of its energy requirements merely in keeping its circuits alive and sensitive; the presence of a message, its functional usefulness or rationality adds only infinitesimally to the total load. Equally adequate, however, are hypotheses found more on traditional biological concepts than on electronic analogues. Thus, when the brain is considered as a great number of functional units, many of which may be reciprocally related with regard to activity, then increased activity in one group of units may result in decreased activity in others. Under such conditions, different functions could result in an altered pattern of distribution of the activity without measurable changes in the net overall oxygen consumption of the brain. Or, even more simply, is it not conceivable that the primitive functions of the brain, namely, the regulation of unconscious vegetative functions in the body, consume so much of the total cerebral oxygen requirements that they obscure the metabolic effects of the later phylogenetic functions found in conscious waking behavior, such as thought and reason?

These studies have not elicited, nor were they designed to elicit information bearing on the more subtle functional, biochemical, or electrical alterations in sleep. They do, however, render untenable those hypotheses which attribute this important phenomenon to an anoxemia, to cerebral ischemia, to narcosis, or to a generalized depression in cerebral metabolism.

SUMMARY

1. Studies of cerebral blood flow, cerebral vascular resistance, and cerebral oxygen consumption, as well as mean arterial blood pressure, hemoglobin concentration, blood gases, and blood pH, were made before or after and during natural sleep in six subjects, during two consecutive determinations under identical conditions in 13 subjects, during a state of fatigue in 25 subjects, and also in 11 normal rested controls.

2. The mean values obtained in the rested subjects were almost identical with the original normal values reported for the method.

3. The fatigued subjects showed no differences from the rested controls except for an elevation in cerebral blood flow which approached statistical significance.

4. During natural sleep there was a statistically significant increase in cerebral blood flow, statistically significant decreases in cerebral vascular resistance and mean arterial blood pressure, and no changes in cerebral oxygen consumption, hemoglobin concentration, and arterial oxygen content.

5. The fatigued subjects who slept were distinguished from those who were unable to sleep by significantly higher values of carbon dioxide tension and lower values of pH in arterial and cerebral venous blood even during the control period. These findings suggest some relationship between respiratory acidosis and the process of falling asleep.

6. The results of these studies make less tenable those hypotheses which attribute sleep to arterial anoxemia, cerebral ischemia, or to a generalized narcosis or other depression in cerebral metabolic rate.

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THE EFFECT OF MENTAL ARITHMETIC ON CEREBRAL CIRCULATION AND METABOLISM[1]

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Considerable evidence exists to suggest that conscious mental activity is coupled to an active utilization of oxygen by the brain. When there is a deficiency in the oxygen supply to the brain, produced, for example, by anoxia or cerebral ischemia, consciousness is rapidly impaired (1, 2). Conversely, the depression of consciousness observed in various pathological states has been found to be accompanied by an appreciable reduction in cerebral oxygen consumption (3–6). Indeed, there has been observed a striking parallelism between the level of conscious activity in these states and the rate of oxygen utilization by the brain (7).

The fact that both cerebral oxygen consumption and conscious mental activity are concomitantly depressed in various non-physiologic states leaves unanswered, however, the question of whether the various mental activities which characterize the state of consciousness require energy derived from oxidative processes. It is conceivable that the oxygen required by the brain to sustain consciousness may be utilized only to maintain its structural and functional integrity without any oxygen being consumed by the actual mental processes themselves, in the sense that muscular work, either skeletal or cardiac, requires additional amounts of oxygen (8, 9). That such may be the case is suggested by the findings of Mangold and his associates that in natural sleep, a state of relative unconsciousness unassociated with pathologic processes, cerebral oxygen consumption is unchanged from the normal waking state (10).

Because of the nature of the phenomena, investigations of mental processes are limited almost exclusively to studies in unanesthetized man. A lack of suitable methods has prevented previous studies of the effects of mental activity on cerebral oxygen consumption. Attempts have been made, however, to describe, at least qualitatively, the effect of such activity on the cerebral circulation. Thus, Fulton has observed in a patient with an arteriovenous aneurysm overlying the occipital lobe that the bruit, audible through the occipital lobe, became more prominent when the patient was reading (11). In ten of fifteen cases, Lennox found increases in the oxygen content of internal jugular venous blood during the reading and performance of problems in mental arithmetic (12). He interpreted these results as indicative of a dilatation of cerebral vessels, reasoning that a decrease in oxygen consumption which would have produced the same findings was less probable.

The present availability of more specific methods permits a more definitive examination of the influence of mental activity on the cerebral circulation and metabolism, the purpose for which this study was undertaken.

METHOD

Studies of the effect of concentrated mental effort on cerebral circulation and metabolism were performed on 13 healthy, young, male university students and faculty members. Seven of the subjects were rested at the time of the study. The remaining six had originally been scheduled for similar studies in natural sleep (10) and had missed four to six hours of sleep; they were used in the present experiments because they were unable to sleep under the conditions of the procedure. Each study consisted of two determinations of cerebral blood flow by means of the nitrous oxide technique (13), first, a control determination during which the subject was in a state of complete physical rest and as great a degree of mental relaxation as the procedure would permit, followed by an experimental determination, during which the subject was presented orally with problems in arithmetic to solve mentally. To allow for a possible

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lag in the effect of mental exercise on the functions measured, the reading of the arithmetical problems was begun five minutes prior to the cerebral blood flow determination and continued throughout the ten-minute period required for the procedure. Since the face mask required by the nitrous oxide technique precluded vocal answers by the subjects, the problems were so designed that the answers were always an integer from one to five which could be indicated on the fingers of one hand. The problems were of sufficient complexity, however, to prevent solution without concentrated mental effort (Figure 1). Each answer, right or wrong, was immediately followed by a new problem. To minimize untoward emotional responses, the subjects were not informed of the correctness of their answers. Simultaneous electroencephalographic recordings from six to eight scalp electrodes were made throughout the study in six of the experiments in order to obtain independent evidence of mental concentration attending the solution of the arithmetical problems.

Mean arterial blood pressure was measured by means of a damped mercury manometer connected to the femoral arterial needle. Blood oxygen and carbon dioxide contents were determined by the manometric technique of Van Slyke and Neill (14). Arterial hemoglobin concentration was measured in the Evelyn photoelectric colorimeter according to a modification of the method of Evelyn and Malloy (15). Blood pH was measured anerobically at room temperature in a Cambridge pH meter and was corrected to 37° C. by means of the factors of Rosenthal (16). Cerebral oxygen consumption, cerebral vascular resistance, and cerebral respiratory quotient were calculated as previously described (13). Blood carbon dioxide tension was computed by means of the nomograms of Peters and Van Slyke (17).

$38 + 19 - 1 \div 7 - 5 = 3$

$\sqrt{26 \times 2 \div 4 + 3} = 4$

$\sqrt{86 - 23 + 1} - 7 = 1$

$\sqrt{2^3 + 4^2 \div 6} = 2$

$11 \times 2 + 20 \div 7 \div 2 = 3$

$\frac{\sqrt{16} \times 5}{10} = 2$

$3^2 + 15 \div 8 = 3$

$\frac{55}{11} \times \frac{3}{\sqrt{9}} = 5$

FIG. 1. SAMPLE PROBLEMS USED IN MENTAL ARITHMETIC STUDY

RESULTS

The data are presented in Tables I and II. Included for comparison are the mean values obtained by the same investigators in a series of control determinations in 11 normal young men and in a series of two consecutive control determinations in 13 "fatigued" individuals (10). The two consecutive control measurements were made under identical conditions but were separated in time by an interval approximating that between the control and experimental determinations in the studies on mental arithmetic (88 and 63 minutes, respectively). Although the subjects in the consecutive control studies were "fatigued" (*e.g.*, had remained awake four to six hours past their usual bedtime at the time of the study), these studies were considered adequate controls for the mental arithmetic studies for two reasons: 1) Six of the 13 subjects used in the studies on mental arithmetic were similarly "fatigued"; 2) no significant differences were found between "fatigued" subjects and normal rested young men (10). In one rested subject (J. Fo.), data on cerebral blood flow, oxygen consumption, and vascular resistance during mental arithmetic are lacking because of technical difficulties in the blood nitrous oxide analyses; the results of the other pertinent measurements which were successfully accomplished are included, however.

The performance of mental arithmetic was accompanied by statistically significant rises in mean arterial blood pressure (MABP) and pulse rate from the control levels (MABP: from 86 to 93 mm. Hg, $p < 0.01$; pulse rate: from 73 to 78 beats per minute, $p < 0.02$). These changes may be related to the moderate degree of apprehension which, at least, according to our subjective observations, many of these subjects exhibited concerning the display of their arithmetical ability. Despite repeated assurances that we were not keeping score, they reacted as though they were taking an examination, and some apologized in advance for any poor showing they might make. Indeed, one subject (E. L.) blocked completely and was unable to answer even the simplest problem. The results in this study were, therefore, ex-

TABLE I

Cerebral blood flow and metabolism during mental arithmetic

Subject	Age	Pulse rate		MABP mm. Hg		CBF cc./100 g./min.		$CMRO_2$ cc./100 g./min.		$(A\text{-}V)O_2$ Vol. %		CVR mm. Hg / cc./100 g./min.		Cerebral R.Q.	
		C	E	C	E	C	E	C	E	C	E	C	E	C	E
Mental arithmetic—13 cases															
J. Fr.†	26	82	96	87	86	76	67	4.2	4.0	5.69	6.05	1.2	1.3	0.94	0.87
S. D.†	22	67	79	99	119	60	43	4.4	3.8	7.24	8.76	1.7	2.8	0.96	0.95
S. K.†	34	67	69	98	108	57	60	3.7	3.6	6.45	5.99	1.7	1.8	0.96	0.95
P. K.†	22	72	85	83	92	62	75	3.4	3.9	5.59	5.23	1.3	1.2	0.95	1.17
R. K.†	22	80	84	87	83	75	71	3.9	3.7	5.23	5.11	1.1	1.2	0.92	1.00
J. Fi.	26	74	73	85	83	73	63	4.2	4.3	5.78	6.76	1.2	1.3	1.05	0.93
C. P.	23	72	68	80	88	103	87	4.9	4.2	4.76	4.82	0.8	1.0	0.96	0.97
I. D.	21	78	76	80	87	64	68	3.7	4.3	5.77	6.34	1.3	1.3	0.98	1.03
F. K.	20	68	78	82	88	58	63	3.8	4.2	6.45	6.63	1.4	1.4	0.99	1.02
J. Fo.	20	68	66	86	94	64	—	3.7	—	5.79	6.25	1.3	—	0.90	0.99
J. A.	24	84	93	86	90	84	81	4.0	4.0	4.80	5.00	1.0	1.1	0.97	0.96
C. S.	24	66	74	83	92	54	57	3.7	4.0	6.78	6.98	1.5	1.6	1.10	0.96
E. L.*†	29	78	78	84	90	57	49	3.2	3.0	5.60	6.19	1.5	1.9	0.98	0.94
Mean	23.7	73.2	78.4	86.3	92.5	69.2	66.8	3.97	4.00	5.86	6.16	1.29	1.45	0.97	0.98
Stand. Error	±1.1	±1.9	±2.8	±1.8	±3.1	±4.0	±3.6	±0.12	±0.07	±0.22	±0.32	±0.08	±0.15	±0.02	±0.02
p‡		<0.02		<0.01		>0.3		>0.9		<0.1>0.05		<0.2>0.1		>0.7	
Consecutive control determinations in 13 fatigued subjects															
		I	II	I	II	I	II	I	II	I	II	I	II	I	II
Mean	23.6	71.0	72.7	90.2	96.0	64.8	60.0	3.65	3.52	5.85	6.02	1.46	1.68	0.93	1.02
Stand. Error	±0.9	±2.1	±2.7	±2.5	±2.1	±5.3	±3.8	±0.21	±0.16	±0.34	±0.29	±0.12	±0.12	±0.03	±0.02
p‡		>0.3		<0.01		>0.1		>0.4		>0.5		~0.01		<0.05	
Normal young men—11 cases															
Mean	21.8	69.3		86.5		54.8		3.34		6.25		1.68		0.92	
Stand. Error	±0.6	±2.6		±1.7		±4.3		±0.23		±0.40		±0.13		±0.03	
p§	>0.1	>0.2		>0.9		<0.05		<0.05		>0.3		<0.02		>0.1	

* Not included in statistical analysis because of complete mental block during mental arithmetic.
† Indicates "fatigued" subjects in mental arithmetic series.
‡ Represents significance of change between two determinations as calculated by method of paired comparisons.
§ Represents significance of difference between normal young men and control values in mental arithmetic series.

TABLE II

Blood constituents during mental arithmetic

Subject	Hb concentrations *Grams %*		O_2 content *Vol. %*				CO_2 content *Vol. %*				pH				CO_2 tension *mm. Hg*			
			Arterial		Int. jugular		Arterial		Int. jugular		Arterial		Int. jugular		Arterial		Int. jugular	
	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
Mental arithmetic—13 cases																		
J. Fr.†	12.4	12.5	17.00	17.33	11.31	11.28	48.29	47.40	53.61	52.65	7.41	7.38	7.34	7.37	39	42	50	48
S. D.†	13.9	14.2	19.10	20.10	11.86	11.34	42.67	42.39	49.60	50.71	7.35	7.39	7.32	7.32	40	37	49	51
S. K.†	13.7	13.5	18.86	18.90	12.41	12.91	44.96	45.73	51.15	51.39	7.31	7.36	7.27	7.30	45	42	56	53
P. K.†	13.9	13.9	18.80	18.82	13.21	13.59	52.28	51.51	57.57	57.65	—	—	—	—	—	—	—	—
R. K.†	14.3	14.2	19.61	19.53	14.38	14.22	47.75	48.67	52.58	53.79	—	—	—	—	—	—	—	—
J. Fi.	15.0	—	19.65	19.80	13.87	13.04	50.30	49.52	56.39	55.83	7.39	7.40	7.32	7.32	44	42	57	56
C. P.	14.2	—	17.66	17.93	12.90	13.11	54.06	53.53	58.61	58.20	7.40	7.38	7.35	7.32	46	46	54	48
I. D.	13.6	—	18.11	18.44	12.34	12.10	51.69	50.97	57.32	57.54	7.39	7.35	7.35	7.31	45	47	53	57
F. K.	14.5	—	19.20	19.43	12.75	12.80	47.20	47.62	53.60	54.40	7.37	7.38	7.32	7.32	43	42	53	54
J. Fo.	15.0	—	19.78	19.93	13.99	13.68	48.17	47.52	53.39	53.68	7.34	7.33	7.29	7.29	47	47	57	58
J. A.	16.1	—	18.90	19.07	14.10	14.07	50.37	48.17	55.03	53.96	7.38	7.42	7.36	7.36	46	40	52	50
C. S.	16.1	—	20.23	20.51	13.36	13.53	49.53	50.12	57.10	56.84	7.41	7.42	7.38	7.38	41	42	51	50
E. L.*†	16.4	16.4	20.20	20.69	14.60	14.50	44.56	44.93	50.06	50.77	—	—	—	—	—	—	—	—
Mean	14.4	13.7	18.91	19.15	13.04	12.97	48.94	48.60	54.66	54.72	7.38	7.38	7.33	7.33	43.6	42.7	53.2	53.5
Stand. Error	±0.3	±0.3	±0.27	±0.27	±0.28	±0.28	±0.91	±0.84	±0.81	±0.72	±0.01	±0.01	±0.01	±0.01	±0.9	±1.0	±0.9	±1.2
p‡	>0.5		<0.02		>0.5		>0.2		>0.7		>0.5		>0.8		>0.9		>0.7	
Consecutive control determinations in 13 fatigued subjects																		
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Mean	14.0	14.1	19.25	19.37	13.46	13.28	48.37	48.07	54.21	54.14	7.41	7.39	7.36	7.34	40.9	41.7	49.5	51.0
Stand. Error	±0.3	±0.3	±0.37	±0.32	±0.27	±0.27	±0.59	±0.51	±0.56	±0.46	±0.01	±0.01	±0.01	±0.01	±1.2	±0.8	±1.3	±0.9
p§	>0.1		>0.3		>0.5		>0.3		>0.8		~0.2		<0.1>0.05		>0.3		<0.05	
Normal young men—11 cases																		
Mean	14.6		19.44		13.20		47.45		53.11		7.39		7.34		41.3		51.6	
Stand. Error	±0.5		±0.49		±0.53		±0.73		±0.60		±0.01		±0.01		±0.9		±1.3	
p§	>0.7		>0.3		>0.7		>0.2		>0.1		>0.3		>0.5		<0.1>0.05		>0.3	

* Not included in statistical analysis because of complete mental block during mental arithmetic.
† Indicates "fatigued" subjects in mental arithmetic series.
‡ Represents significance of change between 2 determinations as calculated by method of paired comparisons.
§ Represents significance of difference between normal young men and control values in mental arithmetic series.

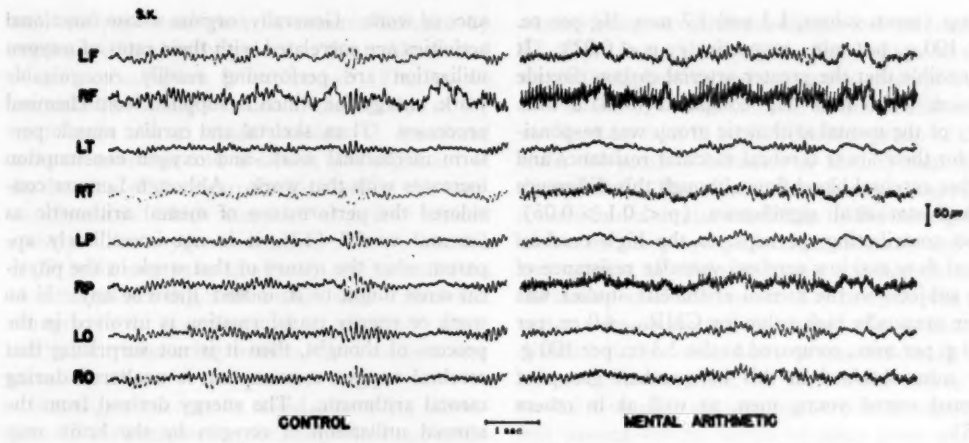

FIG. 2. EFFECT OF MENTAL ARITHMETIC ON ELECTROENCEPHALOGRAPHIC PATTERN

cluded from the statistical analysis. The blood pressure rise during mental arithmetic was not significantly different ($p > 0.9$), however, from a comparable rise in the consecutive control determinations, from 90 to 96 mm. Hg ($p < 0.01$) (Table I). There was no apparent anxiety or apprehension in the latter group to explain this rise, but there were frequent complaints of restlessness and discomfort from lying in one position for so long a period of time. No such complaints were heard in the mental arithmetic studies, perhaps, because of a preoccupation with the arithmetical problems. Unlike the results in the subjects performing mental arithmetic, no significant change in pulse rate occurred in the consecutive controls.

Despite the rise in MABP, cerebral blood flow (CBF) did not change from the control level during mental arithmetic (Table I). The rise in cerebral vascular resistance (CVR) which accompanied these changes was small, however, and lacked statistical significance. A slightly greater but statistically significant increase in CVR was observed in the second of the consecutive control determinations, the mean value being 1.7 mm. Hg per cc. per 100 g. per min. compared to 1.5 mm. Hg per cc. per 100 g. per min. in the first determination ($p \sim 0.01$). Despite the mental effort and despite a shift in the electroencephalographic pattern to higher frequency, lower voltage rhythms during mental arithmetic (Figure 2), both the control and the experimental values for cerebral oxygen consumption (CMR_{O_2}) were identical. Nor was CMR_{O_2} altered significantly in the two consecutive control determinations. Cerebral respiratory quotient (R.Q.) was also unchanged during mental arithmetic although a statistically significant increase from 0.93 in the first to 1.02 in the second of the two consecutive control determinations occurred ($p < 0.05$).

Except for a rise in arterial oxygen content from the control level of 18.9 vol. per cent to 19.2 vol. per cent ($p < 0.02$), no significant changes were found in any of the blood constituents during mental arithmetic. Since no increase in hemoglobin concentration was observed in the five cases included in the statistical analysis in which complete data were obtained, it may be that a hyperventilation associated with the anxiety was responsible. This explanation is questionable, however, in view of the statistically insignificant decrease in arterial CO_2 tension.

A comparison between the control values in the mental arithmetic studies and the results in another control series of normal rested young men reveals some surprising differences. The mean cerebral blood flow, 69 cc. per 100 g. per min., in the control determinations of the mental arithmetic studies significantly exceeded the value, 55 cc. per 100 g. per min., in the independent control series ($p < 0.05$). This difference was associated with a correspondingly lower cerebral vascular resistance in the control period before mental arithmetic than in the independent control

group (mean values, 1.3 and 1.7 mm. Hg per cc. per 100 g. per min., respectively; $p < 0.02$). It is possible that the greater arterial carbon dioxide tension (43.6 mm. Hg compared to 41.3 mm. Hg) of the mental arithmetic group was responsible for their lower cerebral vascular resistance and higher cerebral blood flow although this difference lacked statistical significance ($p < 0.1 > 0.05$). Also contributing, perhaps, to the high cerebral blood flow and low cerebral vascular resistance of the subjects in the mental arithmetic studies was their unusually high value for CMR_{O_2}, 4.0 cc. per 100 g. per min., compared to the 3.3 cc. per 100 g. per min. obtained in this independent group of normal rested young men, as well as in others (13).

Although similar tendencies for high values of CBF and CMR_{O_2} and low values of CVR were observed in the first of the consecutive control determinations in "fatigued" subjects, these values did not differ significantly from those in rested young men. Also, no significant differences were observed between the rested and the "fatigued" groups of subjects within the mental arithmetic series.

DISCUSSION

One of the most intriguing problems in all of neurophysiology is the nature of mental processes, such as thought and reason. Yet, insofar as physiological investigation is concerned, it remains, to a great extent, unexplored territory. In order to approach the problem, we have accepted the common, though by no means universal, notion that mental functions reside within the brain, and the brain represents the proper area for their investigation. Having thus resolved the question of site, we have attempted to determine whether the mental processes involved in the performance of mental arithmetic are of a type in which energy utilization or transformation occurs. A positive result that thought processes require the utilization of additional quantities of oxygen by the brain would have led to such a conclusion. In view of our negative results, however, the conclusions are far less obvious, and we must give careful consideration to a number of possible interpretations.

First of all, it must be pointed out that in the strict physical sense, the performance of a useful function is not synonymous with the performance of work. Generally, organs whose functional activities are correlated with their rates of oxygen utilization are performing readily recognizable work, energy for which is supplied from chemical processes. Thus, skeletal and cardiac muscle perform mechanical work, and oxygen consumption increases with that work. Although Lennox considered the performance of mental arithmetic as "mental work" (12), it is not immediately apparent what the nature of that work in the physical sense might be if, indeed, there be any. If no work or energy transformation is involved in the process of thought, then it is not surprising that cerebral oxygen consumption is unaltered during mental arithmetic. The energy derived from the normal utilization of oxygen by the brain may then serve only to maintain its structural and functional integrity, and none is utilized by the actual thought process itself. The reduction in cerebral oxygen consumption in pathological states of reduced consciousness (7) may then reflect only a block in the maintenance mechanisms, and consciousness is reduced secondarily because of the failure to maintain structural and functional integrity. When there is no interference with the maintenance mechanisms, mental activity may be reduced, as in natural sleep (10), or increased as in mental arithmetic, without any change in cerebral oxygen utilization.

On the other hand, it is impossible to exclude unequivocally the possibility that mental activity consists of energy-dependent processes. Several other reasonable hypotheses which include this possibility can satisfactorily explain our results in mental arithmetic and sleep (10). First, the total energy requirements of the brain, as reflected by the cerebral oxygen consumption, may be independent of mental activity, but biochemical changes associated with that activity may alter the efficiency with which that energy is transformed to a useful form. Since all engines, including biochemical systems, are much less than 100 per cent efficient, considerable variations in energy output can be achieved in this way without altering the total energy supply. Secondly, in view of the complexity of the brain and the existence of inverse relationships between many of its component parts, mental activity or sleep may be accompanied by a redistribution in the patterns of activity throughout it. Areas with increased functional and meta-

bolic activity could then be counter-balanced by areas of reduced activity so that the total cerebral oxygen consumption is unchanged. Finally, we are unaware of any estimate of the fraction of the total brain tissue which consists of functional units or neurones and are even less cognizant of the fraction of the neurones involved in the various functions which comprise conscious mental activity. The oxygen consumption of this fraction of the brain may be so small even under conditions of maximal activity, that we are unable to detect changes in it by means of a relatively crude method for measuring total cerebral oxygen consumption.

Some question may be raised concerning the validity of our controls for the study of mental arithmetic. Since it is impossible for a conscious human being to maintain his mind in a state of complete rest or inactivity, is it reasonable to assume a lesser degree of mental activity in the control determinations than during the performance of mental arithmetic? Evidence to support such an assumption is provided by the electroencephalographic recordings made simultaneously with the determinations in six of the studies. During the control periods a typical resting EEG pattern was generally obtained, but during the mental arithmetic, there was, despite the frequent presence of muscle artifact, a clear and obvious shift to low voltage, high frequency, asynchronous rhythms (Figure 2), a change frequently associated with conditions of presumably increased cerebral activity (18). It appears then that neither the degree of mental activity nor the electroencephalographic pattern are correlated with the total cerebral oxygen consumption.

Our results fail to confirm the rise in internal jugular oxygen content observed by Lennox during mental arithmetic or the elevation of cerebral blood flow which he postulated on the basis of that rise (12). We are unable to account for the discrepancy in results, but in view of the dependence of the internal jugular venous oxygen content on other factors which he did not measure, such as arterial oxygen content and cerebral oxygen consumption, it can scarcely be considered a reliable indicator of cerebral blood flow.

Several unexpected observations in the course of these studies seem to offer information relative to the still controversial question of whether anxiety alters cerebral circulation and metabolism (7, 19). In order to explain the findings of substantially higher control values for cerebral blood flow and oxygen consumption in the mental arithmetic group than in an independent group of controls, the possible influence of anxiety cannot be disregarded. These two series of studies were performed under similar conditions by the same group of workers employing identical methods, and the two groups of subjects chosen at random from the same pool of volunteers. Furthermore, the two series were conducted simultaneously so that individual studies were more or less randomly distributed between them. It is true that five of the mental arithmetic subjects included in the statistics had missed several hours of sleep prior to the study, but a large group of similarly "fatigued" subjects have shown no significant differences from the independent group of normal controls (10). Also within the mental arithmetic series there were no recognizable differences between the "fatigued" and rested subjects. The only immediately apparent difference between the subjects employed in the mental arithmetic studies and the normal controls was in the degree of anxiety exhibited. The subjects in the mental arithmetic studies evidenced considerable apprehension about the showing they would make in solving the problems, not only during the experimental period, but even during the preparations for the study before the control determination. In the absence of any other apparent factor, anxiety must be suspected as a possible cause of the elevated cerebral blood flow and metabolic rate in this group of subjects.

SUMMARY

1. Studies of cerebral circulation and metabolism at rest and during the mental exertion required in solving problems in arithmetic were made in 13 young university students and faculty members.

2. Despite a shift in the electroencephalographic pattern to lower voltage, higher frequency, asynchronous activities and small but statistically significant rises in pulse rate and mean arterial blood pressure, no changes in cerebral blood flow, cerebral oxygen consumption, cerebral vascular resistance, and cerebral R.Q. from the control levels were observed during the performance of mental arithmetic.

3. Except for a slight increase in arterial oxygen content, mental arithmetic was not accompanied by any changes in blood constituents such as oxygen and carbon dioxide concentrations, carbon dioxide tension, pH, or arterial hemoglobin concentration.

4. Comparison between the subjects in the mental arithmetic study and an independent group of normal controls reveals significantly higher values for cerebral blood flow and oxygen consumption and a lower value for cerebral vascular resistance in the mental arithmetic subjects, even during the control determinations. The only immediately apparent difference between the two series which might account for these changes was the obvious anxiety exhibited by the mental arithmetic subjects at exhibiting their arithmetical ability.

5. These findings demonstrate a lack of correlation between both the degree of mental activity and the electroencephalographic pattern and the rate of oxygen consumption of the brain as a whole.

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RENAL HEMODYNAMICS, SODIUM AND WATER EXCRETION IN SUPINE EXERCISING NORMAL AND CARDIAC PATIENTS[1]

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Diminished sodium excretion is one of the factors underlying edema formation in congestive heart failure. The clinical improvement and disappearance of the edema in some cardiac patients treated with bed rest alone suggests the importance of exercise as a potentiating factor in the sodium retention. Exercise studies carried out in normal and cardiac patients in the upright position by other investigators are open to question since the upright position itself is a factor in diminished sodium excretion. In order to exclude postural effects completely, it became necessary to study renal hemodynamics and electrolyte and water excretion in supine exercising subjects.

The present study reports the effect of exercise in the supine subject on renal plasma flow, glomerular filtration rate, sodium and water excretion. Fifteen control subjects and eleven cardiac patients were studied. The control series consisted of convalescent patients who presented no evidence of cardiovascular or renal disease. All of the cardiac subjects were receiving digitalis preparations. All had received mercurial diuretics, varying from one day to two weeks prior to the study. The cardiac subjects were divided into two groups depending on their clinical appraisal at the time of the study. In the "compensated" group were placed those patients who were ambulatory, able to lie flat and were free of edema. In the "severe" group were placed those patients who were confined to bed because of limited exercise tolerance. This group required more frequent mercurial diuretics and were orthopneic. With one exception, they were edematous at time of the study.

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PROCEDURE

All patients were in the post absorptive state and at complete bed rest for one hour or more prior to the study and remained recumbent throughout. Each subject was given sodium phenobarbital, 0.12 gm. subcutaneously, to allay anxiety. All patients were hydrated orally by the administration of 200 cc. water every half hour for one to two hours preceding and during the experiment in order to establish a constant diuresis. Clearance periods were fifteen minutes in duration with two control periods preceding and two recovery periods following the exercise period. Renal plasma flow and filtration rate were determined by clearance methods (Goldring and Chasis [1]). Inulin and sodium para-amino-hippurate were delivered intravenously by means of a constant infusion pump. Urine collections were made through an indwelling catheter, using distilled water and air to insure complete collection. Exercise was accomplished by means of a pedal-resistance type of ergometer set at 10 pounds resistance and attached to the foot of the table. In order to obtain a maximum effort, the subjects were encouraged to pedal as vigorously as possible to the point of exhaustion. A two-minute rest was allowed in the middle of the exercise period to permit the collection of a blood sample. The cardiac subjects required more frequent rest periods because of dyspnea and fatigue. The actual duration of maximal exercise in the control group averaged 8.4 minutes, compared with an average of 8 minutes in the milder cardiac patients and 6.9 minutes in the severe cardiac patients.

Inulin and sodium para-amino-hippurate determinations were done according to the method of Schreiner (2) and Goldring and Chasis (1) and all sodium determinations were made with a Fox internal standard flame photometer.

RESULTS

Each patient served as his own control and the percentage of change from the average of the control values has been derived for each patient for each period. These values were then averaged for each group (Figures 1–3). Changes in renal hemodynamics of 20 per cent or more were considered to be significant.

The results are presented in Table I and summarized for the control subjects in Figure 1. In the control group of fifteen normal subjects, only three showed a significant change in glomerular filtration rate during exercise. Of these, two showed an increase of 22 per cent and 30 per cent and one a fall of 25 per cent. In each of these three subjects Na^+ excretion was also significantly altered and in the same direction as the glomerular filtration rate. Excretion of Na^+ varied considerably in the group, both during control and exercise periods with no consistent directional trend being detected.

Figure 2 represents the averaged data of the five "compensated" cardiac subjects. In this group there was no consistent change in or correlation between glomerular filtration rate and sodium excretion. There was an unexplained decrease in sodium excretion of 44 per cent in one patient which weighted the average for the group and explains the 19 per cent average fall in the sodium excretion shown here. The most marked drop in glomerular filtration rate of any patient in this group was to only 90 per cent of control value or a 10 per cent decrease.

Figure 3 represents data from six patients with severe congestive heart failure. This group re-

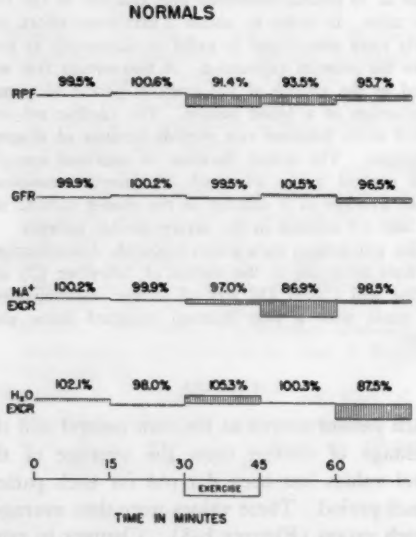

FIG. 1. AVERAGED DATA FOR RENAL PLASMA FLOW, GLOMERULAR FILTRATION RATE, SODIUM AND WATER EXCRETION IN FIFTEEN NORMAL SUBJECTS

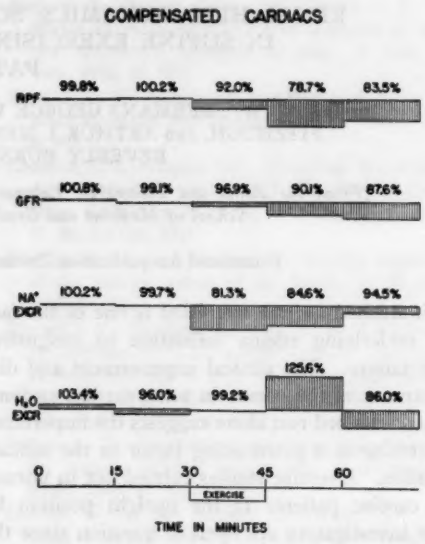

FIG. 2. AVERAGED DATA FOR RENAL PLASMA FLOW, GLOMERULAR FILTRATION RATE, SODIUM AND WATER EXCRETION IN FIVE "COMPENSATED" CARDIAC PATIENTS—SEE TEXT

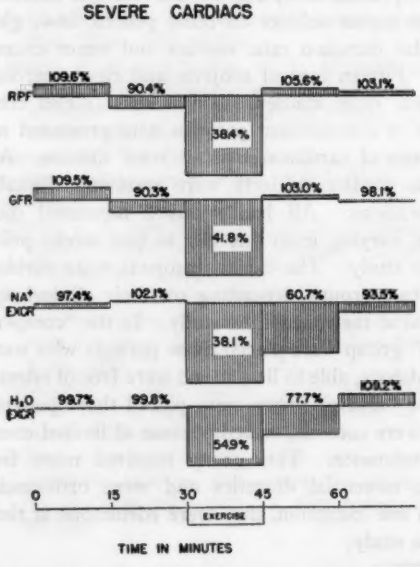

FIG. 3. AVERAGED DATA FOR RENAL PLASMA FLOW, GLOMERULAR FILTRATION RATE, SODIUM AND WATER EXCRETION IN SIX CARDIAC PATIENTS WITH SEVERE CONGESTIVE HEART FAILURE

TABLE I

The effect of exercise upon renal function

Patient	RPF (*cc./min.*)			GFR (*cc./min.*)			Na excretion (*micEq./min.*)			Water excretion (*cc./min.*)		
	Before*	Exercise	After*	Before*	Exercise	After*	Before*	Exercise	After*	Before*	Exercise	After*
						Normal						
M. A.	617	427	617	117	96	100	256	145	193	8.4	5.9	6.5
V. A.	611	474	554	118	117	117	437	421	453	13.6	10.3	8.3
N. B.	617	598	591	105	109	105	88	130	124	10.4	10.4	8.4
P. B.	339	347	369	79	86	90	86	89	95	3.8	3.7	5.7
L. G.	684	579	661	137	140	142	211	163	168	9.3	9.1	9.3
J. L. H.	546	521	438	110	125	94	171	127	123	9.2	10.1	5.4
C. L.	339	301	348	81	69	80	130	96	108	7.5	5.7	6.7
M. Mc.	458	445	478	88	81	86	152	119	122	6.0	5.3	5.6
E. N.	702	453	510	135	101	121	238	179	170	4.5	5.7	0.7
J. S.	821	839	731	133	139	135	135	136	150	11.4	10.1	8.4
O. M. S.	475	467	433	101	101	95	95	98	85	8.6	9.0	7.7
H. W.	596	459	639	131	114	158	169	117	139	8.7	6.2	5.0
N. W.	410	450	362	76	92	76	100	149	105	7.8	9.1	7.6
R. W.	483	584	478	91	118	98	62	75	68	2.0	3.8	5.2
S. G.	406	350	368	106	89	94	136	173	103	4.4	7.1	4.7
						"Compensated" Cardiacs †						
C. M. T.	329	347	260	76	82	64	113	111	82	6.5	6.5	5.3
E. B.	314	304	254	94	95	79	193	159	164	8.2	7.6	5.3
F. J.	582	511	480	95	89	92	124	107	125	8.1	4.5	6.1
F. W.	246	199	179	115	106	98	72	40	49	2.1	3.4	4.7
F. T.	329	293	313	105	94	97	240	201	293	9.0	7.3	7.2
						Severe Cardiacs						
L. P.	257	124	296	108	53	113	68	29	67	.5	.5	.8
J. P.	431	176	367	117	45	112	83	41	80	7.9	2.4	6.9
A. N.	368	179	370	105	56	108	33	11	16	1.3	.6	1.1
J. G.	272	197	241	89	68	89	369	242	257	3.4	5.0	4.3
J. B.	261	28	171	79	10	68	62	10	36	5.9	.5	.6
J. W.	126	12	198	84	18	96	128	29	113	2.9	.4	3.2

* These figures represent the average of two periods.
† See text.

vealed very striking and parallel changes. Every patient in this group had a fall in glomerular filtration rate to well below 70 cc. per min. during exercise whereas no patient in either of the other groups had falls in glomerular filtration rate of that degree. The profound alterations in renal plasma flow and glomerular filtration rate are accompanied by a corresponding diminution in sodium excretion to an average of 38 per cent of control value or an average fall of 62 per cent. During the recovery periods the sodium excretion tended to lag behind glomerular filtration rate in its return toward control values. This would suggest that in exercise, more than one factor may be involved in diminished sodium excretion. It is also of significance that although this group exercised to the best of their ability the work done was less than that of the group with milder failure. The diminished sodium excretion was not always paralleled by decrease in water excretion since in several patients the water excretion actually increased during exercise while the sodium excretion fell.

DISCUSSION

In 1943, Eggleton (3) found a decrease in urinary total nitrogen, chloride and water excretion in medical students during short periods of severe exercise. Later Barclay, Cooke, Kenney, and Nutt (4) measured urine volume, total chloride excretion, renal plasma flow, and glomerular filtration rate (using single dose of inulin or mannitol) in normal subjects during a quarter-mile sprint at full speed. All subjects had a decline in renal plasma flow, glomerular filtration rate, urinary pH and chloride excretion, and there was a fall in urine volume in the majority of subjects. White and Rolf (5) demonstrated decreased re-

nal plasma flow and glomerular filtration rate and marked increases in renal vascular resistance in normal subjects during periods of exercise. Chapman, Henschel, Minckler, Forsgren, and Keys (6, 7) measured renal plasma flow by the constant infusion method and found significant drops during exercise in normal subjects.

Kattus, Sinclair-Smith, Genest, and Newman (8), and Sinclair-Smith, Kattus, Genest, and Newman (9) found a constant fall in sodium excretion without glomerular filtration rate changes during mild upright exercise in both normal and cardiac subjects. The exercise was carried out in most cases after a long period in the supine position or occasionally after a short period of standing with slight motion. These investigators used endogenous creatinine clearances as the measure of glomerular filtration rate in most cases and voided urine collections were made.

One major objection which may be seen in the above studies is the fact that postural changes may have taken place during the exercise period of the experiments. It is well recognized that there is an appreciable diminution in water and electrolyte excretion when the erect position is assumed. This phenomenon has been demonstrated most conclusively in the tilting experiments of Brun, Knudsen, and Raaschou (10, 11). Their data indicate that the anti-diuresis which occurs with tilting to the upright position probably may be explained by a humoral factor such as excessive circulating posterior pituitary secretion. Their patients, however, were motionless and a decrease in circulating blood volume due to venous pooling is likely. Recently, Lewis, Buie, Sevier, and Harrison (12) and Viar, Oliver, Eisenberg, Lombardo, Willis, and Harrison (13) noted that when a subject assumes the sitting position *without exercise,* there is a significant reduction in sodium excretion with no change in glomerular filtration rate as measured by creatinine clearance. These are the same findings that Kattus reports in his exercised subjects.

In the experiments of Kattus, Sinclair-Smith, Genest, and Newman, an attempt was made to separate the postural effects in some subjects by having short control periods of standing with slight motion. Their exhibition of *no change* in renal clearance and slight diminutions in sodium excretion is at variance with preliminary studies done in this laboratory. Experiments reported in this communication were done with the subjects supine throughout, thereby completely ruling out postural effects.

The data here reported differ from those of Kattus, Sinclair-Smith, Genest, and Newman in two important particulars: First, the results show no consistent effect of exercise on sodium excretion or glomerular filtration rate in the normal or "compensated" cardiac subjects, where Kattus found a consistent drop in sodium excretion without any change in glomerular filtration rate; secondly, in the decompensated cardiac subjects there was a profound fall in sodium excretion during exercise and this was invariably accompanied by a comparable reduction in glomerular filtration rate. The reasons for the discrepancies are not immediately apparent, but the consistent fall in sodium excretion in Kattus' experiments may be related to assumption of the upright position rather than to exercise *per se.*

The degree to which alterations in sodium excretion paralleled changes in glomerular filtration during exercise is rather striking in view of the marked variation in sodium excretion in both directions in other periods.

The degree and duration of the urine volume changes with exercise are out of proportion to the changes in renal plasma flow, glomerular filtration rate, and sodium excretion. Water excretion seemed to vary independently of glomerular filtration rate and sodium excretion. Therefore, it seems reasonable to postulate a separate mechanism for the anti-diuresis. Klisiecki, Pickford, Rothschild, and Verney (14) found that the anti-diuresis associated with exercise could not be abolished by complete renal denervation. It may be primarily due to increased formation of anti-diuretic hormone, with neurogenic and hemodynamic factors playing secondary roles.

SUMMARY

A study of renal plasma flow, glomerular filtration rate, sodium and water excretion in fifteen normal and eleven cardiac subjects during supine exercise has been presented. There was no consistent change in or correlation of sodium excretion and glomerular filtration rate in the normal subjects or "compensated" cardiac patients during exercise in the supine position. In the more

severe cardiac patients, renal plasma flow, glomerular filtration rate, and sodium excretion fell significantly in every case during exercise.

It is suggested that the previously reported diminution in Na^+ excretion in exercising cardiac subjects without concomitant reduction in inulin clearance was a postural effect.

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CHANGES IN THE OXYGEN CONTENT OF HEPATIC VENOUS BLOOD DURING EXERCISE IN PATIENTS WITH RHEUMATIC HEART DISEASE

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In a study of the effect of exercise on the cardiac output of patients with rheumatic heart disease (1) small and frequent samples of mixed venous blood were drawn from the pulmonary artery during a period of leg exercise and subsequent recovery. Thus a continuous record of the oxygen saturation of this blood and of the arterio-venous oxygen content difference (A-V difference) was obtained. In most patients abnormally large A-V differences were found during exercise and in some the mixed venous blood was less than 10 per cent saturated. This low saturation suggested that even were oxygen completely extracted from the blood perfusing the exercising limbs, there must also be a considerable reduction of blood flow to other regions of the body. This possibility has been investigated by following changes in the oxygen saturation of venous blood returning from various regions and organs of the body at rest and during exercise, by taking frequent blood samples through an intravenous catheter.

In this paper, the effect of exercise on the oxygen saturation of hepatic venous blood and on the hepatic arterio-venous oxygen content difference (hepatic A-V difference) in patients with rheumatic heart disease is reported. On the assumption that there is little change in the oxygen uptake of the splanchnic region during brief periods of exercise, alterations in the splanchnic blood flow, inversely related to the changes in the hepatic A-V differences, have been inferred.

TABLE I

Clinical details of patients studied

Patient	Sex	Age	S.A. ($m.^2$)	Lesions*	Rhythm†	Disability‡	Operative finding
H1	F	29	1.65	M.S., A.R.	S	2	No operation
H2	M	19	1.78	M.S. & R. A.R.	S	3A	Gross mitral regurgitation
H3	F	40	1.60	M.S. & R. A.R.	S	2	No operation
H4	F	32	1.55	M.S.	S	2	No operation
H5	F	43	1.60	M.S., A.S.	S	3A	No operation
H6	F	32	1.42	M.S., A.R.	S	2	No operation
H7	F	30	1.54	M.S.	S	3A	Severe M.S.
H8	F	34	1.52	M.S.	A.F.	3B	Severe M.S.
H9	F	42	1.59	M.S., A.S.	A.F.	3A	No operation
H10	M	49	1.60	M.S.	A.F.	3B	Severe M.S.
H11	M	26	1.56	M.S. & R. T.R., A.R.	S	3B	Slight M.S.; gross regurgn.
H12	F	52	1.47	M.S. Hypertension	S	3B	Severe M.S.
H13	M	38	1.75	M.S. & R.	A.F.	3B	Mod. M.S.; mod. degree of regurgn.
H14	F	31	1.44	M.R.	A.F.	3B	No operation

* M.S. Mitral stenosis.
M.R. Mitral regurgitation.
A.R. Aortic regurgitation.
A.S. Aortic stenosis.
T.R. Tricuspid regurgitation.
† S. Sinus rhythm.
A.F. Auricular fibrillation.
‡ *Disability grade:* Modified from American Heart Association grading (1).

TABLE II

Cardiac output, intravascular pressures and blood oxygen capacities at rest and during exercise

Patient		Blood oxygen capacity *vol. per cent*	Blood oxygen saturation: Mixed venous *per cent*	Blood oxygen saturation: Arterial *per cent*	A-V diff. *vol. per cent*	Oxygen uptake *ml./min./m.²*	Cardiac index *L./min./m.²*	Pressure (*mm. Hg*): Mean P.A.	Mean P.C.P.	Mean R.A.	Brachia artery
H1	Rest	19.50	72.0	92.0	3.90	176	4.51	42	32	3	—
	Ex.	20.50	44.1	91.7	9.76	573	5.87	47	—	—	—
H2*	Rest	18.44	77.8	96.8	3.52	181	5.16	—	—	4	100/64
	Ex.	19.08	47.7	96.8	9.37	505	5.39	—	—	—	—
H3	Rest	19.47	76.8	92.9	3.14	175	5.58	30	26	2	106/58
	Ex.	20.11	48.3	93.2	9.03	544	6.03	57	—	—	120/64
H4	Rest	17.55	76.4	96.5	3.53	143	4.04	23	12	6	120/66
	Ex.	18.76	41.4	97.6	10.54	701	6.65	—	—	—	150/72
H5	Rest	19.17	79.9	94.0	2.71	139	5.12	18	—	8	124/76
	Ex.	19.78	47.6	95.0	9.38	507	5.41	25	—	—	146/84
H6	Rest	18.70	75.8	95.4	3.74	144	3.86	23	20	6	108/74
	Ex.	19.00	43.9	94.8	9.88	520	5.26	43	—	—	146/84
H7	Rest	18.85	77.7	97.1	3.66	120	3.27	33	24	8	102/62
	Ex.	19.80	51.8	99.2	9.39	484	5.16	59	—	—	122/78
H8*	Rest	14.64	60.1	93.8	4.94	143	2.89	—	—	—	112/56
	Ex.	15.40	40.9	97.0	8.62	423	4.90	—	—	—	—
H9	Rest	20.05	66.0	95.9	6.00	152	2.53	31	20	11	86/54
	Ex.	21.30	25.2	96.0	15.08	630	4.18	60	—	—	130/72
H10	Rest	18.94	54.5	89.5	6.62	144	2.18	50	36	7	—
	Ex.	20.70	21.5	87.7	13.68	435	3.18	80	—	—	—
H11	Rest	17.94	56.3	92.5	6.49	183	2.83	78	32	8	112/78
	Ex.	18.29	21.4	93.1	13.11	410	3.12	90	—	—	120/88
H12	Rest	18.80	69.4	96.0	5.00	169	3.37	57	31	5	220/100
	Ex.	20.03	32.2	95.5	12.68	405	3.19	80	—	—	265/152
H13	Rest	21.02	54.4	94.7	8.47	151	1.79	29	19	8	—
	Ex.	21.80	19.1	97.0	16.98	545	3.21	60	—	—	—
H14	Rest	23.46	61.3	94.7	7.84	146	1.86	36	23	8	—
	Ex.	24.10	22.0	95.2	17.64	492	2.79	40	—	—	—

* In patients H2 and H8 mixed venous blood was sampled from the right atrium.

METHOD

Fourteen patients suffering from rheumatic heart disease, mainly involving the mitral valve, were studied. Patient H12 also had benign hypertension. All patients were under consideration for the operation of mitral valvotomy and their principal clinical features are summarized in Table I. Each study was carried out six hours after the last meal, preparation following the routine described in previous communications (1, 2). After the catheter tip had been introduced into the pulmonary artery and an indwelling needle inserted in the brachial artery, the usual pressure recordings were made and a measurement of the resting cardiac output carried out. The patient then exercised in the supine position on a variable load constant speed bicycle ergometer to a degree near to his tolerance level. Exercise was continued for five minutes in most instances, although five patients were unable to complete this period in the first exercise study owing to dyspnea or weakness. Patient H10, who had previously suffered an embolism of a major artery of the leg, was forced to stop after 3¾ minutes owing to ischemic muscle pain. Measurements of brachial and pulmonic arterial pressures were made throughout exercise and recovery and the cardiac output was measured during the fifth or last minute of exercise in which three samples of arterial and of mixed venous blood were taken and the expired air was collected in a Tissot spirometer, through which expired air had been passing during the earlier minutes of exercise. The validity of such measurements of cardiac output has been previously studied (1).

After the pulmonary artery pressure had returned to resting levels, the catheter was manipulated until its tip lay well in the hepatic vein. Care was taken to ensure that the tip was not impacted and that there were no redundant loops of catheter lying within the right atrium or elsewhere. With these precautions, sampling was possible throughout the subsequent exercise without the catheter becoming displaced from the hepatic vein. Since the hepatic veins join the inferior vena cava separately it is not possible to obtain mixed hepatic venous blood. In the studies here reported, all samples were obtained from the middle of the right lobe of the liver. Bradley (3) has shown that there is relatively uniform extraction of bromsulphthalein from the blood by different areas of the liver and it would appear justifiable to assume that the blood sampled as described was representative of mixed hepatic venous blood.

Thirty minutes after the end of the period of exercise during which cardiac output had been measured, two or three samples of blood were drawn from the catheter in the hepatic vein over a period of one or two minutes. The patient then carried out a second period of exercise, the same degree of work being performed. Most of the patients exercised for five minutes but patients H7 and H10 only completed four minutes, while patients H2, H3, and H11 exercised for ten minutes. One to three blood samples were drawn from the catheter each minute throughout exercise, the time of each sample being noted. Sampling was continued during the recovery period but at a slower rate after the first two or three minutes and was continued during twenty or more minutes of recovery in some patients. Samples of arterial blood were also taken during exercise and recovery in the majority of patients. The position of the catheter tip was checked at the end of the study, although this precaution was omitted in the case of patients H5 and H12.

All blood samples were collected in lightly oiled all glass syringes with heparin filled dead space. A little mercury was then drawn into each syringe which was sealed with a hub cap, shaken and placed on a rotating drum. The percentage oxygen saturation was estimated in duplicate by a spectrophotometric method previously described (4). The blood oxygen capacity at rest and during exercise was estimated by the manometric method of Van Slyke and Neill on blood that had been rotated in a sealed flask in contact with air for fifteen minutes.

Expired air was analyzed by the Scholander micro-method, duplicate analyses being required to check to within 0.03 per cent.

RESULTS

The results of the measurements of cardiac output at rest and on exercise, together with the blood oxygen capacities are given in Table II.

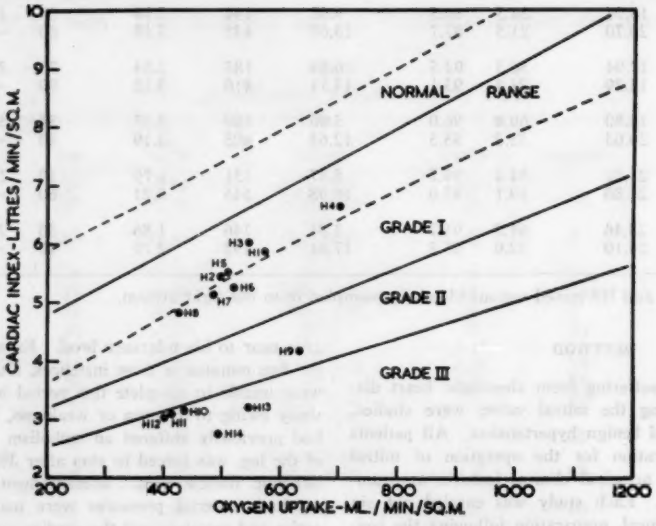

FIG. 1. OXYGEN UPTAKE AND CARDIAC INDEX DURING EXERCISE IN 14 PATIENTS WITH RHEUMATIC HEART DISEASE

The regression line obtained in a previous study from 48 observations on 16 normal subjects is shown together with the 95 per cent confidence limits. Values obtained within the three arbitrarily delineated zones are described as Grades I, II, and III impairment of exercising cardiac output response.

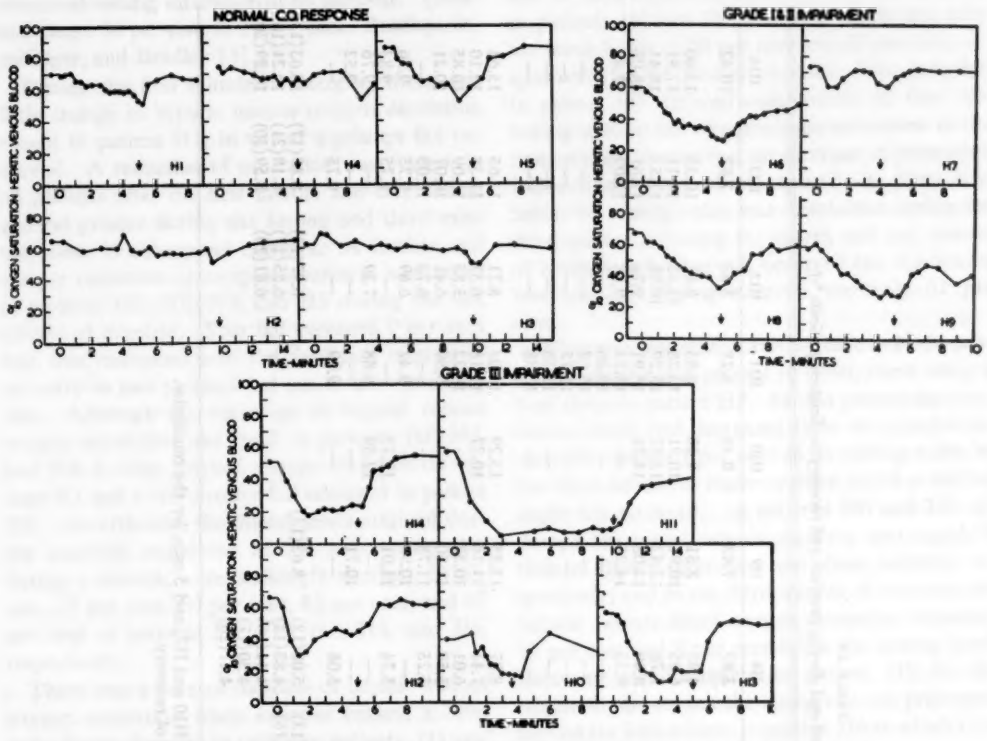

FIG. 2. HEPATIC VENOUS BLOOD OXYGEN SATURATION DURING EXERCISE AND RECOVERY
Exercise commenced at time 0, before which resting values are shown, and ceased at the signal.

The resting and exercise brachial arterial and pulmonary arterial pressure, and the resting pulmonary 'capillary' and right atrial pressures are also given.

Figure 1 illustrates the regression line relating cardiac index to oxygen uptake derived from a previous study of the effect of exercise in normal subjects (2). Its 95 per cent confidence limits are shown by interrupted lines. Three arbitrary zones below the normal range have been delineated and the response of the cardiac output to exercise of each patient here studied is plotted and impairment of response can be conveniently described as Grade I, II, or III.

The Oxygen Saturation of Hepatic Venous Blood at Rest and During Exercise and Recovery

The hepatic venous oxygen saturation of each patient is plotted at rest and during each minute of exercise and the first five minutes of recovery in Figure 2. Changes in arterial oxygen saturation are not shown in this figure, but the resting and exercising values are given in Table II. Some patients were studied during longer periods of recovery than is shown in Figure 2 and details of these findings are given in Table III. The results are considered in four groups according to the grade of the response of the cardiac output of each patient to exercise.

Normal cardiac output response to exercise

Five patients (H1, H2, H3, H4, and H5) fell within this group. The resting hepatic venous saturation in the first four of these patients was 70 per cent, 65 per cent, 67 per cent, and 76 per cent, respectively, although in patient H3 one value at a much lower level was obtained. The fifth patient, H5, had a considerably higher and

TABLE III

Hepatic A-V difference (vols. per cent) at rest and during exercise and recovery in fourteen patients *,†

Min. of study		H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14
Rest		4.25	5.83	5.10	3.63	1.53	6.68	4.35	3.89	7.48	9.34	6.28	5.53	7.82	10.42
Ex.	1	4.67	6.30	5.07	4.33	3.20	7.75	6.28	4.93	8.31	9.40	8.83	8.81	10.33	13.40
	2	5.60	7.12	6.03	4.69	5.24	9.86	6.57	6.35	10.91	10.91	13.28	12.62	15.11	17.81
	3	6.05	7.00	6.07	5.44	6.25	11.04	5.45	7.76	12.42	13.37	15.29	10.94	17.53	18.41
	4	6.62	6.81	6.48	5.74	7.14	11.38	6.34	8.49	14.08	14.74	15.91	9.74	17.81	18.08
	5	7.01	6.91	6.52	6.10	7.83	12.41	—	9.33	14.25	—	15.73	9.88	17.09	17.42
	6	—	7.12	6.74	—	—	—	—	—	—	—	15.46	—	—	—
	7	—	7.65	6.64	—	—	—	—	—	—	—	15.62	—	—	—
	8	—	7.59	6.94	—	—	—	—	—	—	—	15.75	—	—	—
	9	—	7.31	7.00	—	—	—	—	—	—	—	15.38	—	—	—
	10	—	8.01	7.54	—	—	—	—	—	—	—	15.38	—	—	—
Ry.	1	6.50	7.69	8.02	5.89	5.84	12.03	6.89	8.87	13.55	13.29	14.03	8.71	13.65	15.64
	2	5.04	6.93	6.62	5.01	3.30	10.22	5.58	7.44	11.18	10.27	11.18	6.33	11.38	12.10
	3	4.82	6.45	6.42	4.92	2.63	9.73	5.07	6.01	10.57	—	10.22	5.85	10.09	10.85
	4	5.06	—	—	6.72	1.96	9.25	5.05	5.50	11.80	—	9.89	6.41	10.07	10.21
	5	—	—	6.70	7.04	2.04	8.76	5.09	5.25	11.78	—	—	6.73	10.03	—
	6	—	5.90	—	—	—	—	5.21	—	10.99	—	9.42	6.69	9.75	9.76
	7	—	—	7.50	4.93	—	—	5.37	5.74	11.01	12.73	—	—	9.75	9.83
	8	—	—	7.86	—	—	—	—	—	—	12.59	9.88	7.29	—	9.76
	9	—	—	8.06	—	—	—	—	—	10.71	—	—	—	—	9.52
	10	—	—	—	—	—	—	5.25	5.08	—	—	9.99	—	9.75	—
Late recovery								4.79(13) 4.48(16)	5.08(12) 4.88(14) 4.44(16) 4.30(17) 4.17(20)	8.63(13)			6.97(13) 6.85(17) 6.81(20)	8.89(17) 8.18(21) 8.59(30)	10.63(13) 10.21(16) 10.29(20)

* 10 min. exercise performed by H2, H3, and H11, 3½ and 4 min. by H10 and H7, and 5 min. by the remainder.
† During late recovery the figure in parentheses indicates the minute of recovery.

abnormal resting saturation of 86 per cent. (Normal range 56 per cent to 83 per cent: Bradley, Ingelfinger, and Bradley [5].)

During the first minute of exercise, there was little change in hepatic venous oxygen saturation except in patient H5, in whom a prompt fall occurred. A reduction of saturation was evident in all patients after the first minute and this was in general greater during the second and third minutes than in subsequent minutes. A further and steeper reduction of oxygen saturation was noted in patients H1, H2, H3, and H5 during the last minute of exercise. This fall averaged 9 per cent and was continued into the first half minute of recovery in two patients but not in the remaining two. Although the reduction of hepatic venous oxygen saturation was small in patients H2, H3, and H4, a more marked change was seen in patient H1 and a still greater fall occurred in patient H5. Nevertheless, the lowest levels attained during exercise, expressed as the mean saturation during a minute, were strikingly similar; 58 per cent, 55 per cent, 56 per cent, 65 per cent, and 57 per cent in patients H1, H2, H3, H4, and H5, respectively.

There was a prompt increase of hepatic venous oxygen saturation when exercise ceased, a brief delay being observed in only two patients, H1 and H3. Recovery to resting levels was achieved completely, or within 2 per cent, by the second or third minute in all but patient H3. In this patient the saturation remained 6 per cent below resting values during the second and third minute of recovery and subsequently fell further, reaching its lowest value eleven minutes after exercise ceased. In patient H4 also, a second reduction of hepatic venous saturation occurred during recovery.

Grade I impairment of exercise cardiac output

Three patients showed this grade of cardiac output impairment, (H6, H7, and H8). In them, the resting hepatic venous oxygen saturation was 60 per cent, 74 per cent, and 67 per cent, respectively. On exercise there was no delay in the onset of reduction of saturation comparable to that found in the patients with a normal cardiac output response to exercise, although patient H8 showed only a small fall during the first minute. The fall of saturation continued throughout exercise in patients H6 and H8, reaching the lowest minute mean levels of 30 per cent and 37 per cent, respectively, at the end of exercise. The behavior in patient H7 differed considerably in that, following a steep fall of percentage saturation in the first minute of exercise, an increase of saturation followed in the second minute and a level but little below the resting value was maintained during the third minute. During the fourth and last minute of exercise a further fall occurred but the lowest level reached during exercise was only 61 per cent.

When exercise ceased, the increase in blood oxygen saturation was prompt in onset, there being a brief delay in patient H7. In this patient the saturation which had decreased little on exercise returned to within 2 per cent of its resting value by the third minute of recovery after which a further slight fall occurred. In patients H6 and H8, although the initial rate of recovery was rapid, it became slower after two and three minutes, respectively, and in the third minute of recovery the hepatic venous blood oxygen saturation remained 15 per cent and 8 per cent below the resting level. Recovery was complete in patient H8 by the twentieth minute but the study was not prolonged beyond the fifth minute in patient H6 at which time recovery was still incomplete.

Grade II impairment of exercise cardiac output

Only one patient, H9, fell within this grading. After a very brief delay at the onset of exercise, the hepatic venous blood oxygen saturation fell from the resting value of 59 per cent, and, during the last minute of exercise, a steady level around 30 per cent saturation was maintained.

Recovery after exercise was rapid and, by the end of the second minute of recovery, a value only 10 per cent below the resting level had been reached. Thereafter, the percentage saturation fell and rose again, but, even after twelve minutes of recovery, remained somewhat below the resting value.

Grade III impairment of exercise cardiac output

Five patients, H10, H11, H12, H13, and H14 showed this marked impairment of response of cardiac output to exercise. The hepatic venous

blood oxygen saturations at rest were 40 per cent, 58 per cent, 67 per cent, 58 per cent, and 50 per cent, respectively. There was a prompt fall of saturation at the commencement of exercise except in patient H10, in whom no fall was seen until after the first minute of exercise. The fall was more rapid than in the patients in previous groups and the lowest level was reached after 1¼ to 3 minutes of exercise. A rise of saturation then occurred in three of these patients even though exercise was continuing. This rise was relatively small in patients H13 and H14 but was considerable in patient H12 where the mean level in the fifth and last minute was 12 per cent higher than in the second minute of exercise when the lowest saturation was reached.

The lowest minute mean oxygen saturation levels reached were 18 per cent, 6 per cent, 32 per cent, 15 per cent, and 21 per cent in patients H10, H11, H12, H13, and H14, respectively. The level reached in patient H11 is particularly noteworthy, the hepatic venous blood oxygen saturation remaining constant between 6 per cent and 10 per cent from the third to the tenth and last minute of exercise.

When exercise ceased an increase in saturation occurred rapidly in two patients and after a delay of 20 to 30 seconds in the remaining three. Three minutes after exercise had ceased, saturation had reached its resting level in patient H10, was still 3 per cent and 7 per cent below resting in patients H12 and H13, and was slightly higher than resting in patient H14. In patients H10, H12, and H13 there was a subsequent fall in saturation. In H10 it was still 10 per cent below its resting level in the eighth minute of recovery when the study terminated and in H12 it was still 5 per cent below resting level by the twenty-first minute of recovery, but by this time the resting level had been regained in H13. In patient H11 saturation was 20 per cent below its resting value after three minutes of recovery and it remained at this level until the tenth minute when the study ended.

Hepatic and mixed venous blood saturation

The average lowest mean value of oxygen saturation of hepatic venous blood found in any minute during exercise in the group with a normal response of cardiac output was 60 per cent. In the group with Grade I impairment of response it was 44 per cent, in the one patient with Grade II impairment it was 29 per cent, and in the group with Grade III impairment it was 18 per cent.

When the oxygen saturations of blood in the pulmonary artery and hepatic vein were compared in each patient during similar degrees of exercise, it was found that patients with a normal response of cardiac output to exercise showed less unsaturation of hepatic venous blood than of mixed venous blood (mean in hepatic vein, 60 per cent; mean in pulmonary artery, 46 per cent). In the case of two of the three patients with Grade I impairment of response and all five patients with Grade III impairment the reverse was true, the percentage oxygen saturation of mixed venous blood being equal to or higher than that of hepatic venous blood during exercise. In the patients with Grade III impairment the mean oxygen saturation of mixed venous blood was 23 per cent and of hepatic venous blood was 18 per cent.

Hepatic arterio-venous oxygen content difference at rest

The area between the curves obtained when the arterial and hepatic venous oxygen saturation were plotted against time and thus the mean saturation difference for each minute, could be determined from any of the individual studies illustrated in Figure 2. Since the blood oxygen ca-

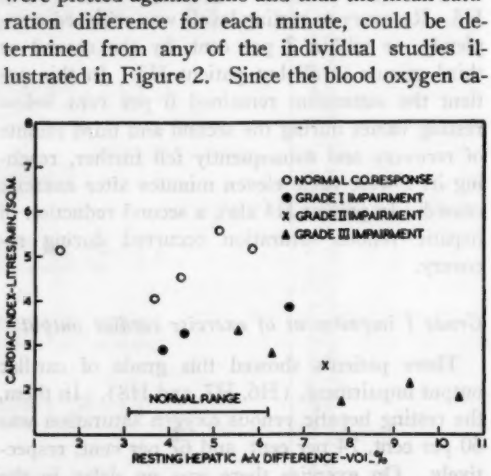

FIG. 3. HEPATIC A-V DIFFERENCE AND CARDIAC INDEX AT REST IN 14 PATIENTS WITH RHEUMATIC HEART DISEASE

The normal range of resting hepatic A-V difference shown is that found by Myers, Brannon, and Holland (6).

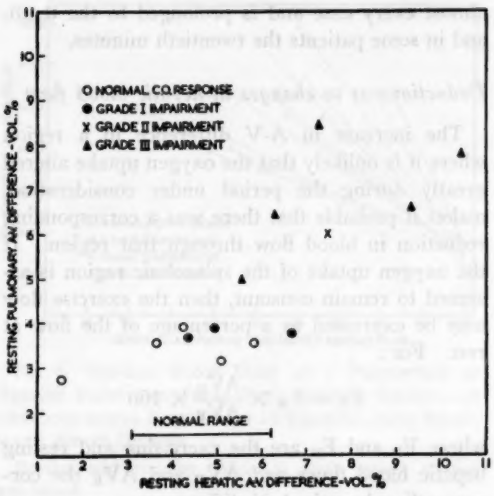
O NORMAL C.O. RESPONSE
● GRADE I IMPAIRMENT
X GRADE II IMPAIRMENT
▲ GRADE III IMPAIRMENT
NORMAL RANGE
RESTING PULMONARY A-V DIFFERENCE - VOL. %
RESTING HEPATIC A-V DIFFERENCE - VOL. %

FIG. 4. HEPATIC A-V DIFFERENCE AND 'PULMONARY A-V DIFFERENCE' AT REST IN 14 PATIENTS WITH RHEUMATIC HEART DISEASE

The normal range of resting hepatic A-V difference shown is that found by Myers, Brannon, and Holland (6).

pacity was known, the mean hepatic arterio-venous oxygen content difference (hepatic A-V difference) could be calculated, not only in the resting state, but during each minute of exercise and recovery. These results are given in Table III.

In Figure 3 the hepatic A-V difference of each patient at rest is plotted against the resting cardiac index. There is a rough linear relationship, low cardiac output in the more disabled patients being associated with a large hepatic A-V difference. In the four patients in whom the resting cardiac index was 2.5 L. per min. per m^2. or less, the hepatic A-V differences were abnormally large. (H9, 7.5 vol. per cent; H10, 9.3 vol. per cent; H13, 7.8 vol. per cent; H14, 10.4 vol. per cent.) The range of normal hepatic A-V difference found by Myers, Brannon, and Holland (6) is 3.1 to 6.2 vol. per cent (49 subjects).

In Figure 4 the hepatic A-V difference for each patient is plotted against the arterial-mixed venous blood oxygen content difference (described as 'pulmonary A-V difference' for convenience). In six patients the hepatic A-V difference and pulmonary A-V difference were approximately equal, while in a further six patients the hepatic A-V difference exceeded the pulmonary A-V difference by an average of more than 2.0 vol. per cent. The hepatic A-V difference was markedly less than the pulmonary A-V difference in only two patients and one of these had an abnormally small hepatic A-V difference.

Hepatic arterio-venous oxygen content difference during exercise

Figure 5 shows the hepatic A-V difference plotted against the total minute oxygen uptake at rest and during exercise, the two points being joined in each case. The exercise hepatic A-V difference plotted is the maximum mean minute value achieved up to the fifth minute of the second period of exercise. The oxygen uptake determined during the first and comparable period of exercise, is taken as a measure of the work performed. In the patients with a normal response of cardiac output to exercise, the hepatic A-V difference increased slightly with exercise just exceeding the upper limits of the normal resting range. A great increase occurred in the patients with Grade III impairment of response of cardiac output although they performed similar or only slightly less exercise. Patients with Grade I and II impairment of response occupy an intermediate position.

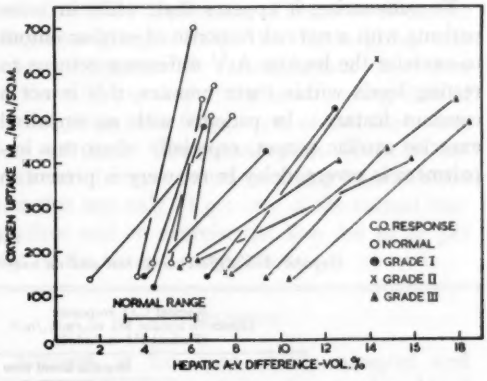

FIG. 5. HEPATIC A-V DIFFERENCE AND TOTAL OXYGEN UPTAKE AT REST AND DURING EXERCISE IN 14 PATIENTS WITH RHEUMATIC HEART DISEASE

The hepatic A-V difference during exercise is the greatest value observed up to the fifth minute of exercise, and the two points are joined in each patient studied. The normal range of resting hepatic A-V difference shown is that found by Myers, Brannon, and Holland (6).

Recovery of hepatic A-V difference after exercise

The studies during recovery are less adequate than those during exercise as the period of continued sampling varied considerably and the greater intervals between sampling makes interpretation less certain.

The hepatic A-V difference had returned to the resting value by the second or third minute in three of the patients (H1, H2, and H5) with a normal cardiac output response to exercise. The remaining patients in this group (H3 and H4) had values which still exceeded the resting hepatic A-V difference in the ninth and seventh minutes of recovery, respectively.

In the patients with Grade III impairment recovery was slower except in patient H14 in whom it was complete by the third minute. The most striking delay in recovery was in patient H11 in whom, after ten minutes, the hepatic A-V difference was still more than 3.0 vol. per cent in excess of the resting value. The hepatic A-V difference was greater than at rest until the seventeenth minute of recovery in patient H13, until the twentieth minute in patient H12 and until the seventh minute in patient H10, when the study ceased. Recovery was delayed similarly in the four patients with Grade I and II impairment of response of cardiac output.

To summarize, it appears that, while in some patients with a normal response of cardiac output to exercise the hepatic A-V difference returns to resting levels within three minutes, this is not a constant feature. In patients with an impaired exercise cardiac output, especially when this impairment is severe, delay in recovery is present in almost every case and is prolonged to the tenth, and in some patients the twentieth minutes.

Deductions as to changes in hepatic blood flow

The increase in A-V difference in a region where it is unlikely that the oxygen uptake altered greatly during the period under consideration, makes it probable that there was a corresponding reduction in blood flow through that region. If the oxygen uptake of the splanchnic region is assumed to remain constant, then the exercise flow may be expressed as a percentage of the flow at rest. For:

$$F_E = F_R \times \frac{AV_R}{AV_E} \times 100$$

where F_E and F_R are the exercising and resting hepatic blood flows and AV_E and AV_R the corresponding hepatic A-V differences.

In Figure 6 the hepatic blood flow during exercise calculated in this way and expressed as a percentage of the patient's resting hepatic flow, is plotted against minute oxygen uptake during exercise. In general the patients with a normal response of cardiac output to exercise show the least reduction of hepatic blood flow. The mean exercising hepatic flow is 59 per cent of the resting flow in this group, as compared with 50 per cent of the resting flow in the patients with Grade III impairment of cardiac output response to exercise. If however these calculations exclude patient H5 whose resting hepatic A-V difference was abnormally small, the mean hepatic blood flow during exercise for the group of patients with normal response of cardiac output was 71 per cent of the resting flow (Table IV).

TABLE IV

Hepatic A-V difference at rest and on exercise and deduced changes in hepatic blood flow *

	Normal C.O. response (Mean O_2 uptake 581 ml./min./m.²) (Patient H5 excluded)			Grade III impairment of C.O. response (Mean O_2 uptake 457 ml./min./m.²)		
		Hepatic blood flow			Hepatic blood flow	
	A-V diff. *Vol. %*	(a) As percentage resting flow	(b) As percentage "mean normal resting flow"	A-V diff. *Vol. %*	(a) As percentage resting flow	(b) As percentage "mean normal resting flow"
Rest	4.70	100	91	7.88	100	54
Exercise	6.64	71	65	15.90	50	27

* Mean values for group with normal cardiac output response to exercise and group with Grade III impairment of exercise cardiac output.

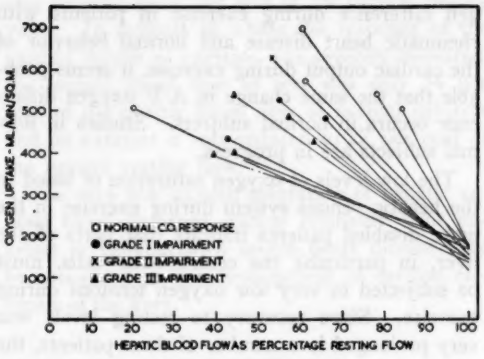
OXYGEN UPTAKE - ML./MIN/SQ.M.
O NORMAL C.O. RESPONSE
● GRADE I IMPAIRMENT
X GRADE II IMPAIRMENT
▲ GRADE III IMPAIRMENT
HEPATIC BLOOD FLOW AS PERCENTAGE RESTING FLOW

FIG. 6. HEPATIC BLOOD FLOW AS A PERCENTAGE OF RESTING FLOW AND TOTAL BODY OXYGEN UPTAKE, AT REST AND DURING EXERCISE, IN 14 PATIENTS WITH RHEUMATIC HEART DISEASE

Resting and exercise values in each patient studied are joined.

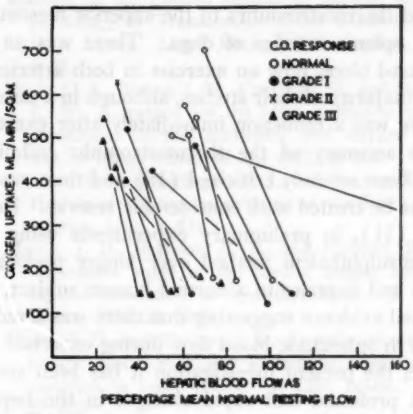

FIG. 7. HEPATIC BLOOD FLOW AS A PERCENTAGE OF MEAN NORMAL RESTING FLOW AND TOTAL OXYGEN UPTAKE, AT REST AND DURING EXERCISE, IN 14 PATIENTS WITH RHEUMATIC HEART DISEASE

The two points are joined in each patient studied.

Since there was considerable variation in resting hepatic A-V differences, many of the values observed being abnormal, a better estimation of the absolute changes in blood flow may be obtained by using, instead of each patient's resting hepatic A-V difference, a mean resting hepatic A-V difference derived from normal subjects. In the largest study of normal subjects yet reported, Myers, Brannon, and Holland (6) obtained a mean resting hepatic A-V difference of 4.29 vol. per cent. Since Myers and Hickam (7) found that the splanchnic oxygen uptake in a group of resting patients in frank congestive cardiac failure was not significantly altered from normal (mean, 41 ml. per min. per m.^{2}: normal mean, 38 ml. per min. per m.^{2}), it can be assumed that the resting splanchnic oxygen uptake of the patients here studied was also equal to the mean normal resting value. Thus the resting hepatic blood flow can be expressed as a percentage of the mean resting hepatic blood flow of normal subjects. For:

$$F_R = F_{MNR} \times \frac{4.29}{AV_R} \times 100$$

where F_R is the patient's resting hepatic blood flow, AV_R his resting hepatic A-V difference and F_{MNR} is the mean normal resting hepatic blood flow. If it is further assumed that the splanchnic oxygen uptake of the patients was unaltered by exercise, the hepatic blood flow on exercise can also be derived as a percentage of the mean normal resting hepatic flow by the same relationship.

The values for all patients at rest and on exercise obtained by making these assumptions are shown in Figure 7, the two points for each patient being joined. Mean values for the group with normal response of cardiac output to exercise, again omitting patient H5, and for the group with Grade III impairment of response of cardiac output are given in Table IV. In the group with a normal response the resting hepatic blood flow fell from 91 per cent of the mean resting flow of normal subjects to 65 per cent of this flow when they exercised. The patients with Grade III impairment of response had a resting hepatic blood flow that was only 54 per cent of the normal resting flow and on exercise the flow fell to 27 per cent of the normal resting flow.

DISCUSSION

Although it has been widely accepted and taught that there is a reduction in splanchnic blood flow during exercise, there is little convincing evidence in support of this. Barcroft and Florey (8) observed contraction of the exteriorized spleen in dogs during exercise together with very transient blanching of a part of exteriorized colon. Herrick, Grindley, Baldes, and Mann (9) ap-

plied thermostromuhrs to the superior mesenteric and splenic arteries of dogs. There was an increased blood flow on exercise in both arteries in the majority of their studies, although in a number there was a reduction immediately after exercise. The accuracy of the thermostromuhr technique has been severely criticized (10) and these results must be treated with considerable reserve. Bradley (11), in preliminary experiments using the bromsulphthalein method over longer periods of rest and exercise in a normal human subject, obtained evidence suggesting that there was a reduction in splanchnic blood flow during exercise.

In the present investigation it has been shown that profound and rapid changes in the hepatic A-V difference occurred in patients with rheumatic heart disease during leg exercise in the supine position and that these changes were greater in patients with severe limitation of cardiac output response to exercise. Unless there were quite extraordinary and unexpected increases in splanchnic oxygen uptake during exercise, this suggests that the hepatic blood flow of these patients, which was already considerably reduced at rest, was still further reduced during exercise to about one quarter of the normal resting flow. Thus on the basis of a mean normal resting hepatic blood flow of 1530 ml. per min., as determined by Bradley, Ingelfinger, and Bradley (5), the hepatic blood flow during exercise in the patients with a normal cardiac output response was of the order of 1 liter per min. Similarly, the patients with Grade III impairment of cardiac output had a mean resting flow of about 800 ml. per min. which fell on exercise to approximately 400 ml. per min. These findings at rest in the patients with Grade III impairment are in close agreement with the results of Myers and Hickam (7) whose patients were in congestive failure.

If on exercise this reduction in blood flow to non-exercising parts of the body did not occur in these patients with low unchanging cardiac outputs, then the maintenance of the blood pressure (see Table II) in the presence of considerable vasodilation in the legs, and of adequate blood supply and oxygen transport to the exercising muscles would not be possible.

The changes in hepatic A-V oxygen difference during exercise in normal subjects are not known but, in view of the definite increase of A-V oxygen difference during exercise in patients with rheumatic heart disease and normal behavior of the cardiac output during exercise, it seems probable that the same change in A-V oxygen difference occurs in normal subjects. Studies in normal subjects are in progress.

The low levels of oxygen saturation of blood in the hepatic venous system during exercise in the more disabled patients indicate that parts of the liver, in particular the centrilobular cells, must be subjected to very low oxygen tensions during exercise. Since recovery to resting levels was very prolonged in a number of these patients, the oxygen tensions in the centrilobular cells must have been abnormally low for considerable periods of time. Centrilobular necrosis is a well recognized result of anoxia (12) and it has also been shown that recurrent attacks of minor centrilobular necrosis may lead to fibrosis. The degree of exertion in these experiments, which did not cause dyspnea in many cases, must be paralleled not infrequently in the everyday life of these patients. It would appear possible, therefore, that many of the more disabled patients with heart disease suffer from repeated, marked and prolonged reduction of splanchnic blood flow and that this may play a part in the causation of the disturbances of liver structure and function found in a number of such patients.

SUMMARY

The changes in oxygen saturation of hepatic venous blood have been followed by frequent catheter sampling during and after leg exercise in patients with rheumatic heart disease.

The hepatic oxygen A-V differences were found to increase in all patients during exercise, the increase being greatest in the patients in whom the response of the cardiac output to exercise was impaired. In some of these disabled patients when exercise ceased the return of the hepatic A-V difference to its resting level was delayed for twenty minutes or longer.

On the assumption that the splanchnic oxygen uptake of these patients was normal at rest and did not change during exercise, it has been deduced that the hepatic blood flow of the patients with a normal response of cardiac output to exercise was almost normal at rest and on exercise

was reduced to 65 per cent of the normal resting value. In patients in whom the response of the cardiac output to exercise was severely impaired, the hepatic blood flow at rest was only 54 per cent of the resting blood flow of normal subjects and on exercise it was reduced to 27 per cent of the normal resting flow.

The very low oxygen saturation of hepatic venous blood observed during relatively light exercise in patients with severe heart disease may be concerned in the development of cardiac cirrhosis of the liver.

ACKNOWLEDGMENTS

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BIOSYNTHETIC DETERMINATION WITH RADIOACTIVE SULFUR OF TURN-OVER RATES OF VARIOUS PLASMA PROTEINS IN NORMAL AND CIRRHOTIC MAN [1, 2]

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Data concerning the life-span of various human plasma proteins are yet meager. Methods have been difficult and data often uncertain of interpretation. Approximations have depended chiefly upon determining the rate of release of isotopic labels from protein molecules. Both *in vitro* chemical incorporation of isotope (I^{131}) and *in vivo* biosynthetic labeling (N^{15}, S^{35}) have been employed.

In this laboratory, an effort has been made to measure the "life-duration" of total serum proteins, albumin, fibrinogen, beta-1 lipoproteins, and gamma globulins in normal and cirrhotic man. Biosynthetic introduction of the radioisotope tag (S^{35}) has been chosen rather than chemical labeling so as to avoid any alteration in biologic life-span which might result from chemical labeling procedures. Two types of experiments have been carried out: measurement of rate of isotope loss in plasma protein fractions (I) after feeding S^{35}-labeled cystine and (II) following transfusion of plasma previously labeled by a normal human donor fed S^{35} cystine. A very considerable effort has been made to utilize the newer and more precise chemical and physical methods in separating the various plasma proteins from small blood samples. Relatively homogeneous protein fractions have been isolated for isotope analysis. In a few experiments, simultaneous observations have been made upon rates of albumin decay with both biosynthetic (S^{35}) and chemical (I^{131}) labeling methods. Studies have been conducted simultaneously in normal subjects and in subjects with advanced portal cirrhosis having gross abnormalities of plasma proteins, with hope of discovering if differences are present in the biologic life-span of plasma protein components.

METHODS

Subjects Chosen for Study

Normal subjects

Individuals were male laboratory colleagues or medical students under 36 years. All were stable in weight, had good health, and showed normal bromsulfalein excretion, thymol turbidity tests and serum electrophoretic patterns. Regularity of the individual's usual high protein diet and working hours was maintained throughout the period of study. Serum total protein, albumin concentrations, hematocrit, and body weight remained constant during the period of observations.

Cirrhotic subjects

These subjects were selected for study as being in an advanced stationary phase of portal cirrhosis. The majority had been confined to the chronic disease unit of King County Hospital for many weeks before commencing observations. Although gross alteration in the concentration of various plasma proteins with marked hypoalbuminemia was present in most, nearly all were edema-free at the time of study. Serum protein concentrations, hematocrit, and body weight were constant during the period of study. All cirrhotic subjects except one [5] were confined to the Research Metabolic Ward throughout the period of observation, and all were maintained on constant diet (calories to maintain weight, protein 90 gm., fat 90 to 130 gm., sodium restricted as necessary to avoid edema). Detailed data of each defining the severity of cirrhosis, body weight, and caloric intake [6] are given in Table I; brief case summaries appear in the Appendix.

[1] This investigation was supported by research grants from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service (A-173); the John and Mary R. Markle Foundation; the A. H. Robins Company, Richmond, Va.; and funds for Research in Biology and Medicine accruing through Initiative 171 of the State of Washington.

[2] Presented in part before the annual meeting of the Western Society for Clinical Research, January 29, 1954.

[3] John and Mary R. Markle Scholar.

[4] A. H. Robins Research Fellow in Gastroenterology.

[5] Subject 11, studied in chronic disease unit.

[6] Though variations in caloric and protein intake relative to body weight occurred, these were not considered to be of the magnitude shown to influence significantly rates of plasma protein metabolism (1).

Isotope Preparation and Administration

Radioactive sulfur

Dose and form of S^{35} given to each subject are shown in Table II. In early experiments, chemically synthesized S^{35}-labeled dl-cystine [7] was used. In later observations, S^{35}-labeled l-cystine [8] was employed; this was prepared by the action of yeast upon labeled sulfate, with subsequent chromatographic isolation of the amino acid.

Oral administration of cystine: The dose of amino acid, contained in 50 to 150 ml. of very dilute hydrochloric acid, was taken by the subject intermittently during breakfast.

Plasma transfer: Normal donors compatible for plasma transfer to the selected recipients were bled in the fasting state 20 hours after oral administration of S^{35}-labeled cystine, utilizing the facilities of the King County Central Blood Bank.[9] Approximately 825 ml. of whole blood were collected in two bottles, each containing 120 ml. standard acid citrate dextrose [10] solution. After storage at 4° C. for 1 hour, the bottles were centrifuged, and the plasma separated into two labeled-plasma units of approximately equal volume. In cases where the recipient was of other than the major blood group of the donor, though agglutinin titres were very low, 10 ml. Sharp & Dohme Solution Blood Group A and B Specific (Witebsky) Substances were added to a plasma unit. Duplicate plasma units were promptly transfused (within 3 hours of bleeding) into a normal and a cirrhotic subject. Erythrocytes were resuspended in saline and returned to the donor.

Radioactive iodine (I^{131})

The iodinated human albumin preparation [11] was reported to contain 1 to 2 iodine atoms per molecule of protein, with a concentration of 10 mg. protein and 45 μc I^{131} per ml. Exactly 102.25 μc were injected with rinsing into the rubber tubing of the intravenous set conveying S^{35}-labeled plasma (or saline) to each subject. Thyroid uptake of radioactive iodine was blocked by administering a saturated solution of potassium iodide, 10 drops three times daily, throughout the period of the study.

Separation of Plasma Protein Components

Collection of blood

About three times weekly, 90 to 100 ml. blood were drawn in the fasting state. Thirty ml. were used for fibrinogen preparation, and serum was obtained from the

TABLE I

*Data on cirrhotics at time of study**

Case	Hepatomegaly	Splenomegaly	Jaundice	Ascites	Peripheral edema	Spider angiomata	Esoph. varices	Serum TP *gm. %*	Serum alb. *gm. %*	Prothr. *% normal conc.*	Total serum bilirubin *mg. %*	BSP % retention 45 min. *5 mg./Kg.*	Cephalin flocc. *48 hrs.*	Thymol turbidity *ml. $BaSO_4$*	Liver biopsy obtained	Wt. of patient *Kg.*	Caloric intake†
11	0	0	0	0	0	0	Porta-caval shunt for varices	7.29	3.49[e]	80	0.4	8	3+	2.0	+	49	2,200
12	+	0	0	0	0	+	0[r]	8.00	2.64[e]	40	0.5	26	4+	1.1	+	52	2,400
13	+	+	slight	v. slight	v. slight	0	0[r]	6.25	2.04[e]	50	2.3				+	61	2,200
14	+	+	slight	0	0	+	0[r]	6.98	2.62[a]	73			3+	2.6	+	82	2,400
15	+	+	slight	0	v. slight	+	+[r]	7.54	2.83[a]	28	3.5		4+	3.7	0	81	2,700
16	+	+	0	0	0	0	0[r]	7.54	2.88[a]	26	1.0		4+		0	58.5	2,100
17	+	+	slight	0	0	0	+[r]	8.30	3.20[a]	57	3.2	29	4+		0	91.5	2,300
18	+	+	slight	0	0	+	0[r$_{Es}$]	7.80	2.89[a]	40	1.5	22	4+	4.4	+	61.5	2,400

* r = roentgen examination; Es = esophagoscopy; e = free electrophoretic determination; c = chemical determination.
† Contained 90 gm. protein daily.

[7] Generously provided by Dr. Harold Tarver, Dept. Physiological Chemistry, University of California.

[8] Chiefly obtained from Dr. Harold Tarver; some furnished by Abbott Laboratories.

[9] The authors are grateful for the assistance of Dr. Richard S. Czajkowski and Mr. Soren Juul.

[10] Trisodium citrate 22.0 gm., citric acid 8.0 gm., dextrose 24.5 gm. per liter.

[11] Abbott Laboratories, administered within four days of iodination.

TABLE II

Isotope dosage and excretion

Sulfur (S^{35})

Subject	Oral dose Form	Oral dose mg.	Oral dose mc.*	IV dose Plasma-citrate ml.	Conc. total prot. gm. %	Total radio-activity mc. $\times 10^3$	Donor source S^{35} plasma Subject No.	Urine excretion % total dose Day 1	Urine excretion % total dose Days 1–3	Fecal excretion % total dose Days 1–3
				Normal subjects						
1	dl-cystine	10.8	0.587					57.0	60.4	1.9
2	dl-cystine	6.6	0.739					19.4	20.5	1.2
3	l-cystine	36.0	1.600					16.0	22.7	0.5
4				300	5.8	4.93	3			
5	l-cystine	81.0	3.214					12.9	17.1	
6				260	5.5	12.19	5			
7	l-cystine	79.0	2.217					14.0	18.7	
8	l-cystine	79.0	2.218					11.0	13.5	
9	l-cystine	140.0	4.965							
10				255	5.1	14.90	9			
				Cirrhosis						
11	dl-cystine	19.7	0.712					13.7	15.5	1.9
12	dl-cystine	19.5	0.706					15.4	16.8	2.4
13	dl-cystine	7.2	0.540					22.4	27.3	3.1
14	l-cystine	20.0	0.890					7.1	12.5	0.4
15				235	5.8	4.16	3			
16				270	5.2	12.31	5			
17	l-cystine	79.0	2.171							
18				255	4.5	14.10	9			

Iodine (I^{131})

(Each subject received IV 2.7 mg. human serum albumin containing 102.25 μc I^{131}*)

Subject	Urinary excretion % total dose Day 1	Urinary excretion % total dose Days 1–3
	Normal	
9	8.2	20.2
10	8.8	20.9
	Cirrhosis	
18	6.5	18.1

* Approval of this dosage was obtained from the Subcommittee on Human Applications of the Atomic Energy Commission.

remainder after allowing it to clot for 1 to 2 hours. One part of ACD solution (2) was added to three parts of serum; this serum solution was then allowed to stand overnight at 4 to 5° C.

Fibrinogen

Employing the general method of Surgenor, Alexander, Goldstein, and Schmid (3), calcium was removed from 30 ml. of blood by passage over sodium cycle Dowex-50 cation-exchange resin. One-tenth volume of barium sulfate was suspended in the plasma, which was then cooled to 0 to 1° C. After stirring for 10 minutes, the barium sulfate was removed. The fibrinogen was then precipitated by diluting the plasma with an equal volume of acetate-ethanol reagent [12] to give a final pH of 6.9 and ethanol concentration of 6 per cent. After standing one hour at 4 to 5° C., the precipitate was separated by centrifugation at 0° C., washed with 5 ml. cold 6 per cent ethanol in pH 6.8 phosphate buffer (ionic strength 0.01), and recentrifuged. This washing procedure was repeated three times.

Albumin

ACD serum was carried through the Cohn Method 10 fractionation (2) to obtain Fraction IV + V. This

[12] The quantity of pH 4 acetate buffer (0.16 M sodium acetate, 0.08 M acetic acid) to be added was determined by titration of a plasma aliquot to pH 6.9.

product was subjected to further differential precipitation and extraction in buffered ethanol solutions at − 5° C., as devised by Batchelor and Brown (4) to yield Fraction V; this allowed separation and discard of a glycoprotein by means of differential solubility.

Beta-1 lipoproteins

ACD serum was fractionated by the Cohn Method 10 (2) to obtain Fraction I + III. In the later experiments, this sample was then subjected to preparative ultracentrifugation in low and high density salt solutions, in accord with the general plan of Lindgren, Elliott, and Gofman (5): The Cohn I + III paste from 15 ml. of serum was dissolved in 9 ml. cold 0.1 M sodium chloride (density 1.004[13]), and centrifuged in a 10.5 ml. capacity lusteroid tube at 4° C. for 12 hours at 40,000 rpm (Model L Spinco Ultracentrifuge, size 40 head). The top 7 ml. of fluid which contained chylomicrons, lipids, and lipoproteins of S_f values approximately 17 to 70 was discarded and the walls of the tube wiped clean. To the residual solution was added, with stirring, 7 ml. cold saline-glycine reagent, density 1.090[14] (0.3 M sodium chloride, 3.38 M glycine) to obtain a final density of 1.064.[15] After centrifugation for 12 hours at 4° C. and 40,000 rpm, 1 to 2 ml. of the floating viscous yellow layer plus saline was carefully removed with a glass capillary or short-beveled needle and syringe from the interface of the tube wall, lipoprotein and air. This material was presumed to contain lipids and lipoproteins of S_f values approximately 2 to 17, thus lipoproteins migrating electrophoretically with the beta globulins and of density greater than 1.004 but less than 1.064. This lipoprotein solution was then dialyzed against running tap water for 6 hours before oxidizing to inorganic sulfate.

Gamma globulins

ACD serum was separated by the Cohn Method 10 (2) to obtain Fraction II. For subjects 9, 10, and 18, a slight modification suggested by Russ (6) was employed; this attempted more complete removal of beta globulins by increasing their solubility through use of larger volumes of reagents (7 plasma volumes instead of 5). In a few experiments, Fraction II was subjected also to further purification by a modification of the starch zone electrophoresis method of Kunkel and Slater (7), using a pH 9.0 phosphate buffer, ionic strength 0.1; eluted protein from the trailing half of the gamma globulin zone, presumed to be free of beta globulins, was analyzed for sulfur specific activity. Neither of these modifications, however, appeared to alter the character of the isotope data finally obtained.

Appraisal of Homogeneity of Protein Fractions

One or two samples of each fraction from most experimental subjects were analyzed for electrophoretic homogeneity. The electrophoresis apparatus (Perkin-Elmer Model 38) was used with diethyl-barbiturate buffer, pH 8.6, ionic strength 0.1 and sample protein concentrations of 1 to 1.5 per cent. Photographs were taken with the knife-edge and Schlieren lens-scanning system. Analyses of enlarged electrophoretic patterns were made by dropping perpendiculars to the base line from the low point between peaks. The pattern areas were then obtained by taking the mean of the planimeter-measured areas beneath the upper and lower contour edges of the descending limb patterns (8). The β anomaly was analyzed by measuring the area above the base line and subtracting from it the area below the base line. Per cent electrophoretic homogeneity was found as follows: albumin: normal 85.4 to 100.0 (average 91.9), cirrhosis 79.6 to 91.1 (average 85.6); β_1 lipoprotein: normal 87.9 to 100.0 (average 94.2), cirrhosis 80.3 to 100.0 (average 90.1); fibrinogen: normal 69.6 to 100.0 (average 81.7), cirrhosis 56.3 to 88.8 (average 77.5); γ globulin:[16] normal 81.2 to 89.1 (average 84.7), cirrhosis 77.8 to 94.5 (average 88.7). Electrophoretic mobility of fibrinogen peaks ranged from − 2.0 to − 2.6 (average − 2.3) $cm.^2\ volt^{-1}\ sec.^{-1} \times 10^{-5}$.

Ultracentrifugal analyses of a number of beta-1 lipoprotein preparations were made[17] in the analytical ultracentrifuge (Spinco Model E) equipped with a diagonal bar, cylindrical lens Schlieren optical system and analytical rotor (type A); this was operated at 24° C., 52,640 rpm with an acceleration time of 6 minutes and camera exposures taken at 0, 8, 16, 24, 32, and 40 minutes. In each case only one discrete migrating boundary was observed (Figure 1) with the flotation constant shown in Table III; however, in a few instances a very small amount of a slightly faster component could be detected on the leading edge of the major boundary.

The beta-1 lipoproteins were further characterized by the chemical analyses summarized in Table III. Total and free cholesterol[18] were determined by the method of Sperry and Webb (12), phospholipid[18] by a modification of the combined methods of Whitehorn (13) and Fiske and Subbarow (14), and nitrogen as described below.

The purity of the fibrinogen preparations, measured as clottable protein by Morrison's procedure (15), was found to be 55 to 63 per cent.

[13] At 23° C.

[14] At 23° C.

[15] Calculated at 23° C. without considering the effect of the lipoprotein; at 5° C. this density was determined to be 1.069. For subjects 9, 10, and 18, sufficient reagent was added to obtain a final *measured* density of 1.064 at 23° C.; the sample was then made to volume by adding reagent of same density.

[16] Whereas the major peak of other fractions was always symmetrical, the gamma peak was usually asymmetric, with a steeper gradient on the trailing edge.

[17] Kindly performed by Mr. Roger Wade of the Department of Biochemistry.

[18] For these determinations we are indebted to Mrs. Della Ramsden of the Department of Pathology.

FIG. 1. ULTRACENTRIFUGAL FLOTATION PATTERN OF REPRESENTATIVE β_1 LIPOPROTEIN SAMPLE (SUBJECT 17), EXPOSURES AT 16 AND 32 MINUTES

Sample centrifuged at 52,640 rpm at 25° C. in sodium chloride-glycine solution of approximate density 1.064.

Preparation of Samples for Isotope Analysis

S^{35} analysis

Plasma proteins: Protein sulfur was first oxidized to inorganic sulfate by the method of Pirie (16) and then crystallized as the benzidine sulfate (17). Protein samples of 50 to 150 mg. were oxidized with 5 ml. Pirie's reagent by being warmed slowly to 280 to 300° C. and kept at this temperature for 18 hours. After removal of the residual perchlorate by heating, 1 ml. conc. HCl was added and the sample was reheated at 300° in the air bath until dry. After cooling, 3 ml. water and 3 drops of 1 N HCl were added; any precipitate remaining after slight warming was filtered off. Two ml. of benzidine dihydrochloride reagent (2 per cent in 0.2 N HCl) were added and the solution was then kept at 4° C. for 24 hours. When the sample was small, crystallization was aided by the addition of ethanol to 50 per cent concentration, shaking and reducing the temperature to 0 to −5° C.

Approximately 4 mg. quantities [19] of benzidine sulfate were collected (sample diameter 21 mm.) on Whatman 42 filter paper by filtration on a stainless steel screen or sintered glass apparatus similar to that used by Tarver and Schmidt (18) and Kinsell and co-workers (19). The precipitate was washed with cold distilled water and cold 95 per cent ethanol. After drying at room temperature, the paper was mounted for counting upon a Tracerlab E-7 brass disc with ring. The total quantity of sulfur counted as benzidine sulfate was determined by titrating an aqueous solution of the benzidine sulfate (boiled frequently to remove CO_2) with 0.01 N NaOH using phenol red as an indicator.

Urine: Small aliquots (0.2 ml.) of the diluted (1:10) 24-hour urine collections were evaporated and counted [20] on copper or stainless steel planchets.

Feces: Since the total isotope content of benzidine sulfate from stool samples was low, only filtered aqueous extracts of 24-hour stool samples were evaporated and counted (without self-absorption correction) on stainless steel planchets.

I^{131} analysis

Serum: Two ml. samples of serum were counted in 4 ml. screw-top glass vials.

Urine: Two ml. aliquots of 24-hour urine specimens were similarly counted. For collections made 28 to 35 days after the injection of I^{131} albumin, urine was concentrated 5 to 10 fold before counting.

Isotope Counting

S^{35} counting

S^{35} activity was measured in a windowless gas-flow Geiger-Müller counter having an efficiency of 50 per cent and a background of 15 counts per minute.

[19] These varied from 0.18 to 1.82 mg. benzidine sulfate per cm.^{2} sample surface. The smaller amounts were from β_1 lipoprotein fractions, the highest from albumin. The amounts of plasma or serum required to furnish benzidine sulfate samples of adequate size varied widely according to the specific protein fraction, and depended chiefly upon the plasma concentration and the sulfur content of the protein component. The benzidine sulfate plated upon a single planchet represented the recovered amounts of the individual proteins from the following quantities of serum or plasma: albumin 0.8 to 1.5 ml. serum, gamma globulin 1.7 to 7 ml. serum, β_1 lipoprotein 22 to 30 ml. serum, fibrinogen 15 to 20 ml. plasma, total protein 0.4 ml. serum. Thus, whereas duplicate samples of albumin, gamma globulin, and total protein were customarily prepared, only single planchet samples of fibrinogen and β_1 lipoprotein were obtained for each experimental point.

[20] Several control experiments showed that no self-absorption correction need be applied.

Corrections were applied to the counting data as necessary for self-absorption, coincidence (20), and decay, Day-to-day variation, and differences in instrumentation, were eliminated by counting a C^{14} reference standard with all samples.

I^{131} counting

I^{131} activity was measured in a well-type sodium iodide crystal scintillation counter having an efficiency of 28 per cent and a background of 80 to 100 counts per minute. Samples were prepared and counted in duplicate except for the concentrated urine samples.

Corrections for both day-to-day variations in counter sensitivity and isotopic decay were made by counting an I^{131} standard source daily, then correcting the sample activities by a factor which was the ratio of I^{131} standard source initial activity to I^{131} standard source daily activity.

Differential counting

There was no interference of either of the isotope activities with the counting of the other. I^{131} was found to be completely removed from S^{35} during the protein digestion and benzidine sulfate crystallization while the scintillation counter could detect only the I^{131} activity of the isotope mixture.

Miscellaneous Analytical Procedures

Plasma volume

The method of Gibson and Evans (21) was used, where 5 ml. of 0.5 per cent Evans Blue dye (T-1824) [21] were injected, 4 to 6 heparinized blood samples being withdrawn between 10 and 25 minutes after dye injection. Following determination of plasma dye concentrations in a Beckman Model B Spectrophotometer (wave length 605 mμ), these data were plotted against time on linear graph paper, and the curve extrapolated to obtain zero time plasma dye concentration.

Nitrogen

Micro-Kjeldahl digestion was employed, using the mercury catalyst of Hiller, Plazin, and Van Slyke (22), followed by distillation of ammonia from the Kirk apparatus (23) into 2 per cent boric acid solution, and semimicrotitration of the distillate with standard sulfuric acid. Corrections for non-protein nitrogen were made (24).

Serum total protein concentration

The factor 6.25 was employed to convert grams of nitrogen to total protein.

Albumin concentration

Chemical determination was conducted by salting-out the globulins by the method of Reinhold, Steward, and Gilman (25, 26); the nitrogen of the albumin filtrate was measured by micro-Kjeldahl as described above.

[21] William R. Warner & Co.

Plasma fibrinogen concentration

The method of Ratnoff and Menzie (27) was modified by using the colorimetric determination of protein content described by Lowry, Rosebrough, Farr, and Randall (28). For converting tyrosine to fibrinogen concentrations, the average factor used was determined by three experiments to be 7.38 with a standard deviation from the mean of 0.14.

Serum beta-1 lipoprotein concentration

This was roughly approximated from dried weight (9) of total sample.

Experimental errors

The errors of the various specific activities used in making half-life determinations were: (a) *Plating of benzidine sulfate.* Samples of less than 0.4 mg. per cm.[2]

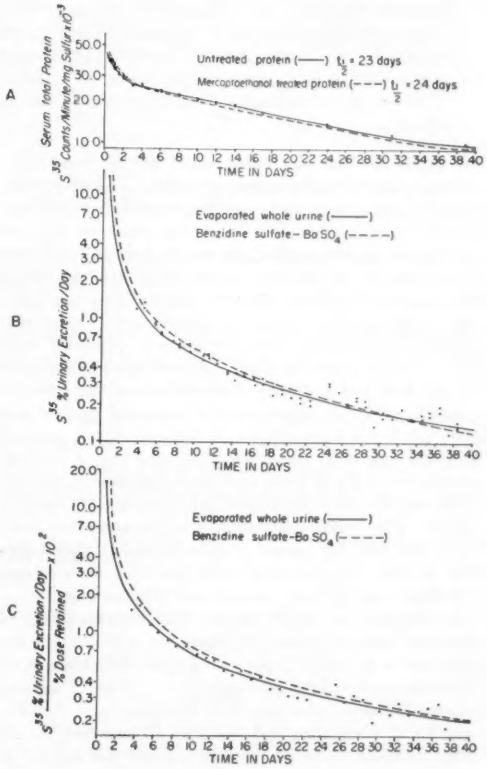
A
Serum Total Protein
S^{35} Counts/Minute/mg Sulfur x 10^3
Untreated protein (——) $t_{\frac{1}{2}}$ = 23 days
Mercaptoethanol treated protein (– – –) $t_{\frac{1}{2}}$ = 24 days
TIME IN DAYS
B
S^{35} % Urinary Excretion/Day
Evaporated whole urine (——)
Benzidine sulfate– $BaSO_4$ (– – –)
TIME IN DAYS
C
S^{35} % Urinary Excretion/Day / % Dose Retained x 10^2
Evaporated whole urine (——)
Benzidine sulfate–$BaSO_4$ (– – –)
TIME IN DAYS

FIG. 2. SUBJECT 7: STUDIES OF URINARY S^{35} EXCRETION AND STABILITY OF PROTEIN-BOUND ISOTOPE

Daily isotope excretion shown as the broken lines in graphs B and C was obtained as the product of sulfur specific activity from isolated benzidine sulfate times total sulfur excreted, determined as barium sulfate. Solid lines are drawn through experimental points shown.

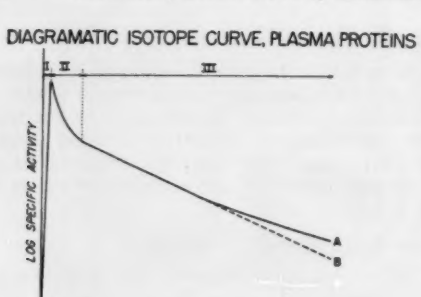

FIG. 3. CURVE A: FRACTIONS OF β_1 LIPOPROTEIN AND FIBRINOGEN OBTAINED FOLLOWING ORAL ADMINISTRATION OF S^{35}-LABELED CYSTINE OR AFTER TRANSFUSING S^{35}-LABELED PLASMA

CURVE B: FRACTIONS OF γ GLOBULINS ISOLATED FOLLOWING TRANSFUSION OF S^{35}-LABELED PLASMA, AND ALBUMIN OR TOTAL SERUM PROTEINS OBTAINED AFTER EITHER ORAL ADMINISTRATION OF S^{35}-LABELED CYSTINE OR INTRAVENOUS TRANSFER OF S^{35}-LABELED PLASMA

(β_1 lipoprotein samples only) could not be evenly distributed over the entire planchet surface. Since self absorption correction factors are based upon even distribution, a standard deviation from the mean of ± 2 per cent would be introduced if the sample were to cover only 60 per cent of the planchet surface area. (b) *Counting.* The standard deviation from the mean of counting rates, due to the random nature of disintegration, were calculated from curves constructed from published data (20). These errors for the different specific activities on the first and last days involved in the half-life calculation were, for experiments where oral cystine was given: ± 1 and 3 per cent for β_1 lipoprotein, ± 1 per cent for other fractions; and when S^{35}-labeled plasma was administered: ± 3 and 9 per cent for β_1 lipoprotein, ± 2 and 5 per cent for other fractions. (c) *Titration of benzidine sulfate.* The standard deviation from the mean was ± 0.3 to 1.0 per cent for normal γ globulin and all β_1 lipoprotein samples, but less than ± 0.5 per cent for cirrhotic γ globulin and all total protein and albumin samples.

Considering the above factors, the resultant standard deviation from the mean of values for half-life was determined to be ± 1 to 2 per cent for all protein fractions following oral cystine administration. After S^{35}-labeled plasma infusions, this standard deviation was ± 6 per cent for β_1 lipoprotein and ± 2.5 to 3.5 per cent for all other fractions. The law of least squares was applied to determine the trend of the experimental points whenever a straight line was drawn for a half-life calculation.

RESULTS

Multiple plasma protein fractions were studied in seven normal and five cirrhotic subjects fed S^{35}-labeled cystine, and in three normal and three cirrhotic patients infused with S^{35}-labeled plasma. Summary data concerning the form of isotope administered, dosage, and gross fecal and urine excretion in the various subjects are presented in Table II. The S^{35} label was excreted mainly in the urine; large proportions were lost during the first two to three days, after which excretion gradually diminished but continued for many weeks (Figure 2). The proportion of total oral dose of isotope present at 24 hours in the rapidly circulating total plasma proteins[22] was: for subject 3, 4.1 per cent; for subject 5, 5.0 per cent; and for subject 9, 6.7 per cent.

General form of isotope curve

Although the form of the isotope concentration curves of the plasma protein fractions studied was

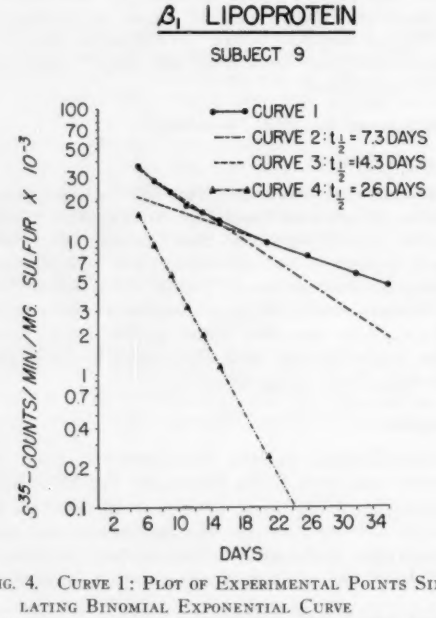

FIG. 4. CURVE 1: PLOT OF EXPERIMENTAL POINTS SIMULATING BINOMIAL EXPONENTIAL CURVE

CURVE 2: STRAIGHT LINE DRAWN THROUGH EXPERIMENTAL POINTS OF DAYS 4 TO 14, GIVING APPARENT $t_{1/2}$ OF EARLY PORTION PHASE III

CURVE 3: SLOW EXPONENTIAL COMPONENT OBTAINED BY EXTRAPOLATION OF TERMINAL PORTION OF CURVE 1

CURVE 4: FAST EXPONENTIAL COMPONENT OBTAINED BY GRAPHIC SUBTRACTION OF CURVE 3 FROM INITIAL PORTION OF CURVE 1

[22] Those contained in the plasma space, as defined with Evans blue dye.

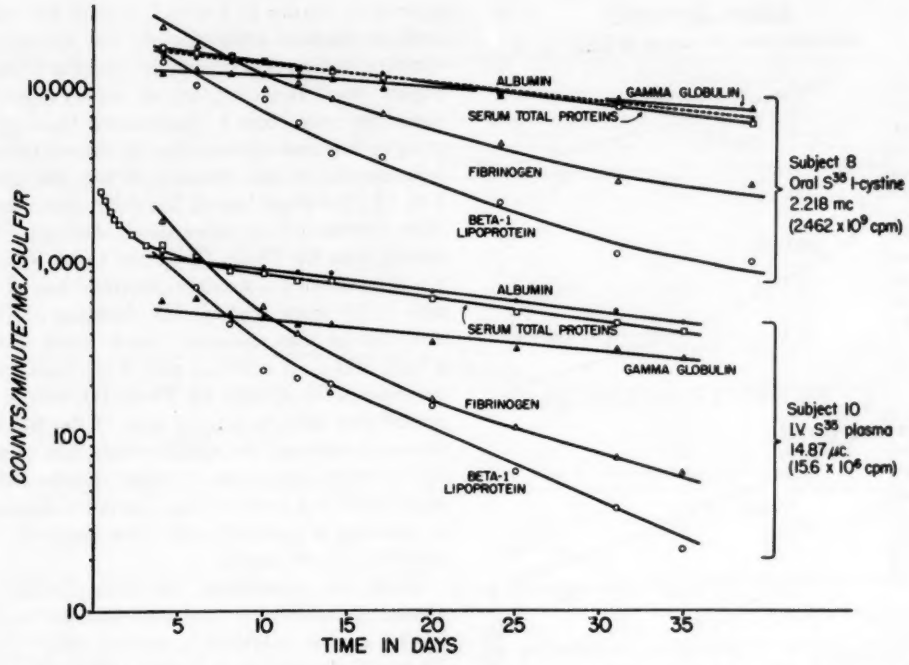

FIG. 5. DATA CURVES OF ALL PROTEIN COMPONENTS STUDIED IN A REPRESENTATIVE SUBJECT FROM EACH TYPE OF EXPERIMENT CONDUCTED

found to be similar to that previously described by others (29–31), certain consistent differences between specific fractions were noted. The plot of the log of specific activity of the sulfur against time for each protein fraction could be divided into the three general phases shown in Figure 3: Phase I—*Formation and release of labeled plasma proteins into the vascular system.* Following oral administration of S^{35}-labeled cystine, the duration of this phase varied from 16 to 22 hours. This phase was absent following the intravenous administration of labeled plasma. Phase II—*Equilibration and dilution of labeled plasma proteins with unlabeled proteins of the body's fluid*

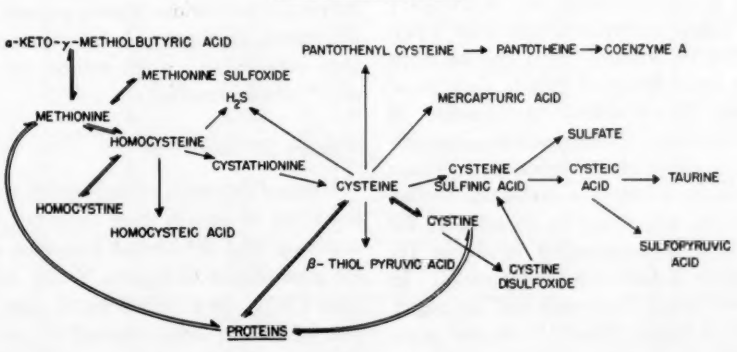

FIGURE 6

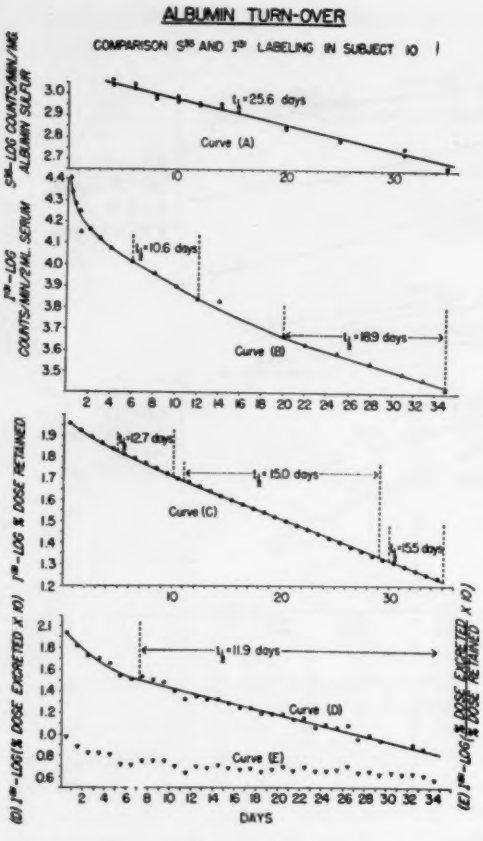

FIGURE 7

pools. Metabolic degradations of course occur simultaneously throughout this period, which has been analyzed by Forker and Chaikoff (32), Schoenberger, Kroll, Sakamoto, and Kark (33) and Berson, Yalow, Schreiber, and Post (34). Phase II varied in duration from one to eight days, in most cases being of two to four days' length. Phase III—*Metabolic degradation of labeled plasma proteins.* Differences between protein fractions in curve shape appeared in Phase III. For albumin, a steady exponential decline of specific activity was found in all subjects for the total duration of observation in Phase III (Curve B, Figure 3, Curve A in Figure 7). In contrast, for both beta-1 lipoprotein and fibrinogen, a semi-log plot of data in Phase III seemed to assume the shape of an exponential binomial curve (Curve A, Figure 3; Curve 1, Figure 4), which could be resolved arbitrarily into two exponential components by graphic analysis (Curves 3 and 4, Figure 4). However, in all observations on fibrinogen and beta-1 lipoprotein fractions a straight line could reasonably be drawn through experimental points obtained during the period 4 to 11 days after isotope administration; thereafter, specific activity more slowly declined. The plotted data for Phase III of the Cohn Fraction I + III exhibited a steady exponential loss of isotope in all cases studied; the duration of these observations was, however, much more limited (Table VII). A semi-log plot of the total serum protein specific activity for Phase III usually appeared as a straight line, in spite of the fact that the curve reflected the varied metabolism of multiple protein components; in many instances there appeared to be a greater delay (up to 8 to 12 days) in reaching a constant slope than occurred with albumin (1 to 4 days).

With few exceptions, the data plotted for gamma globulins after oral administration of S^{35}-labeled cystine exhibited a marked delay (often 10 to 14 days) before appreciable decrease of specific activity began. Thereafter, a steady exponential loss of isotope occurred. Thus, in this group of subjects, when the duration of observations was less than 14 days, little apparent turnover was observed (Table VIII). This was evident in spite of the fact that this fraction appeared maximally labeled within 24 hours of isotope administration. In contrast, subjects receiving donor-labeled plasma exhibited no unusual delay before showing a uniform decline of isotope in this component. The characteristic isotope die-away curves for the various plasma protein fractions are illustrated in Figure 5 by representative data plots obtained in a single subject for each method of S^{35} administration.

Urinary S^{35} excretion

Urinary excretion of radioactive sulfur was followed for 40 days in three subjects (7, 8, and 17) receiving oral S^{35}-labeled l-cystine, according to the general plan of Berson, Yalow, Schreiber, and Post (34). In a typical set of data, relating per cent of isotope dose excreted to per cent of administered isotope retained (Figure 2), a uniform

TABLE III

Characterization beta-1 lipoprotein fraction

Subject	S_f	Electrophoretic mobility $cm.^2\ volt^{-1}\ sec.^{-1} \times 10^{-5}$	Chemical analysis: Total cholesterol / Nitrogen	Free cholesterol / Total cholesterol	Total cholesterol / Phospholipid	Nitrogen % Dried weight*
		Normals				
1	4.38	−3.60	7.14	0.26	1.43	—
5	—	−3.47	—	—	—	3.88
6	—	−3.36	—	—	—	4.34
7	5.66	−3.82	—	0.29	1.35	—
8	4.36	−3.35	—	0.27	1.43	—
9	4.28	—	7.73	0.28	1.37	—
10	4.59	—	7.47	0.27	1.29	4.86
P.G.	—	—	8.76	0.25	1.35	3.60
P.G.	—	—	9.46	0.24	1.45	3.54
		Cirrhosis				
15	4.41	−3.97	8.33	0.42	1.46	—
16	—	−3.22	—	—	—	4.08
17	6.20	—	—	0.40	1.03	—
18	4.34	—	8.29	0.33	1.31	—
IX	—	—	6.94	0.42	1.19	3.89
Reference values						
Oncley, Gurd, and Melin (9)			7.36	0.27	1.05	4.2
Armstrong, Budka, and Morrison (61)		−3.9 to −4.3				
Russ, Eder, and Barr (10)					1.35	
Gofman, Jones, Lindgren, Lyon, Elliott, and Strisower (11)	3–8 (26° C.)					

* Obtained by the directions of Oncley, Gurd, and Melin (9).

TABLE IV

Albumin turn-over *

Method S^{35} administration	Subject	Weight *Kg.*	Plasma volume *ml.*	Albumin conc. *gm./100 ml. serum*	Total plasma albumin *gm.*	Plasma albumin *gm./Kg. body wt.*	Apparent half-life: S^{35} $t_{\frac{1}{2}}$ days	Apparent half-life: I^{131} slowest component $t_{\frac{1}{2}}$ days	Total duration observations *days*
				Normal subjects					
Oral	1	68	4,120	4.05[c]	166.9	2.45	25.8		9
S^{35}	2	84	2,632	3.95[c]	104.0	1.24	44.3		11
Cystine	3	77.3	3,068	4.46[e]	136.8	1.77	23.4		15
	5	70	2,667	4.18[e]	111.5	1.59	26.7		30
	7	70.5	3,922	4.54[e]	178.1	2.52	27.4		39
	8	74.1	3,489	4.15[e]	144.8	1.95	27.4		39
	9	86	4,762	4.38[e]	208.6	2.42	38.8	15.6	36
IV	4	73.6	3,388	4.16[e]	140.9	1.91	16.6		14
S^{35}	6	61.4	3,041	4.54[e]	138.1	2.65	22.3		29
Plasma	10	100	4,808	4.75[e]	228.4	2.28	25.6	18.9	35
				Cirrhosis					
Oral	11	58	3,589	3.49[e]	125.3	2.16	25.4		11
S^{35}	12	52	4,356	2.64[c]	115.0	2.21	21.8		10
Cystine	17	91.5	3,840	3.20[e]	122.9	1.34	62.6		39
IV	15	80	5,910	2.83[e]	167.3	2.09	24.5		14
S^{35}	16	58.5	4,124	2.88[e]	118.8	2.03	23.0		29
Plasma	18	61.5	4,098	2.89[e]	118.4	1.92	26.1	24.9	35

* e = free electrophoretic determination; c = chemical determination.

TABLE V

Fibrinogen turn-over

Method isotope administration	Subject	Fibrinogen conc. *mg./100 ml. plasma*	Apparent half-life, Graphic analysis No. 1*		Determined $t_{\frac{1}{2}}$ components, Graphic analysis No. 2†		Total duration observations *days*
			$t_{\frac{1}{2}}$ days	Period $t_{\frac{1}{2}}$ calc. days after intake isotope	Fast $t_{\frac{1}{2}}$ days	Slow $t_{\frac{1}{2}}$ days	
			Normal subjects				
Oral	3	—	5.6	5–12	1.7	10.0	15
S^{35}	5	293	5.9	5–12	3.5	20.7	30
Cystine	7	279	6.7	4–14	4.7	24.3	39
	8	286	8.0	4–17	5.0	31.4	39
	9	269	7.1	4–14	4.5	25.8	36
IV	4	—	4.2	4–14	—	—	14
S^{35}	6	251	3.4	4–11	2.7	18.0	29
Plasma	10	276	3.7	4–12	2.1	10.6	31
			Cirrhosis				
Oral	14	—	10.7	2–14	—	—	14
S^{35}							
Cystine	17	245	10.9	4–17	6.2	26.0	39
IV	15	—	4.7	4–14	—	—	14
S^{35}	16	251	4.0	4–11	2.8	31.7	29
Plasma	18	357	5.2	5–15	3.3	24.8	35

* As per Curve 2 of Figure 4.

† As per Curves 3 and 4 of Figure 4; approximately 80 to 85 per cent contribution by fast component when extrapolated to zero time.

TABLE VI

β_1 lipoprotein turn-over

Method isotope administration	Subject	β_1 lipoprotein conc. *mg./100 ml. serum*	Apparent half-life, Graphic analysis No. 1*		Determined $t_{\frac{1}{2}}$ components, Graphic analysis No. 2†		Total duration observations *days*
			$t_{\frac{1}{2}}$ days	Period $t_{\frac{1}{2}}$ calc. days after intake isotope	Fast $t_{\frac{1}{2}}$ days	Slow $t_{\frac{1}{2}}$ days	
			Normal subjects				
Oral	3	—	3.1	3–8	11.4	10.8	15
S^{35}	5	330	4.8	5–12	2.5	12.2	30
Cystine	7	250	7.4	4–17	4.5	63.1	39
	8	300	6.3	4–14	4.2	16.5	39
	9	—	6.1	5–11	3.5	13.7	36
IV	4	—	3.8	4–14	1.6	6.5	14
S^{35}	6	170	3.0	4–16	2.1	10.0	29
Plasma	10	150	2.9	4–10	1.0	7.3	35
			Cirrhosis				
Oral							
S^{35}	17	280	10.0	6–14	4.2	21.0	39
Cystine							
IV	15	—	6.1	2–11	—	—	11
S^{35}	16	220	3.4	5–11	2.2	15.5	29
Plasma	18	—	5.0	4–12	1.6	9.8	35

* As per Curve 2 of Figure 4.

† As per Curves 3 and 4 of Figure 4; approximately 80 to 85 per cent contribution by fast component when extrapolated to zero time.

TABLE VII

Turn-over Cohn I+III fraction *

Subject	Oral S^{35} cystine: $t_{\frac{1}{2}}$ days	Period $t_{\frac{1}{2}}$ calc. days following intake isotope	Total duration observations *days*
	Normal subjects		
1	6.2	2–9	9
2	7.8	2–11	11
	Cirrhosis		
11	11.1	3–11	11
12	8.9	2–9	9
13	10.4	1.5–11	11

* About 60 per cent of this fraction is β_1 lipoprotein.

daily per cent excretion of isotope was not observed. This would seem expected inasmuch as urinary excretion of isotope should reflect the widely differing rates of catabolism of many varieties of body proteins labeled by S^{35} cystine, as well as reutilization of isotope among all compounds of the body's organic sulfur pool. Since the amount of isotope administered by the infusion of labeled plasma was small, it did not seem possible to study accurately S^{35} excretion over a similarly prolonged period in these cases.

TABLE VIII

Turn-over gamma globulins *

Method isotope administration	Subject	Apparent half-life $t_{\frac{1}{2}}$ days	Total duration observations *days*	Concentration γ globulins *gm./100 ml. serum*
		Normal subjects		
Oral S^{35} Cystine	1	∞	9	1.1[e]
	2	71	11	1.2[e]
	7	48	39	0.80[e]
	8	49	39	0.87[e]
	9	85	36	0.73[e]
IV S^{35} Plasma	4	19.7	14	0.67[e]
	6	25	29	0.94[e]
	10	35	35	0.80[e]
		Cirrhosis		
Oral S^{35} Cystine	11	150	9	1.2[e]
	12	130	9	2.1[e]
	13	51	11	1.9[e]
	14	151	14	1.99[e]
	17	31	39	3.10[e]
IV S^{35} Plasma	16	40	29	2.70[e]
	18	51	35	2.47[e]

* c = chemical determination (36); e = free electrophoretic determination.

Turn-over data, various protein fractions

From the summary of turn-over data for albumin in Tables IV and X, it appears that the apparent turn-over time is slightly shorter for normal subjects infused with S^{35}-labeled plasma than for those fed S^{35}-labeled cystine. No clear differences between the S^{35} data for normal subjects and patients with cirrhosis are observed.

The turn-over curves for I^{131}-labeled albumin indicated the presence of components of varying half-times, similar to the observations of Berson, Yalow, Schreiber, and Post (34). In each subject, the slowest component observed after 8 to 14 days, yielded a half-life value of the same range as reported by Berson, Yalow, Schreiber, and Post for "satisfactorily" iodinated albumins. Early high urinary excretion of I^{131} reflected the presence of rapidly degraded components in the injected albumin preparation (Table II).

In fibrinogen and beta-1 lipoprotein fractions, as with albumin, there appeared to be a somewhat longer apparent half-life in subjects fed labeled amino acid precursor as compared with values obtained following injection of S^{35}-labeled plasma (Tables V and VI). The data suggest a slightly

TABLE IX

Turn-over serum total proteins

Method isotope administration	Subject	Total protein conc. *gm./100 ml. serum*	Apparent half-life days	Period $t_{\frac{1}{2}}$ calc. days following intake isotope	Total duration observations *days*
		Normal subjects			
Oral S^{35} Cystine	1	7.48	14.4	1–9	9
	2	7.57	30.1	3–11	11
	3	7.15	20.4	5–15	15
	7	7.39	23.5	8–31	39
	8	7.91	23.8	4–39	39
	9	6.91	38.8	4–36	36
IV S^{35} Plasma	4	7.27	12.7	4–14	14
	10	7.52	23.5	8–35	35
		Cirrhosis			
Oral S^{35} Cystine	11	7.29	20.0	3–11	11
	12	8.00	17.7	1–9	9
	13	6.25	24.4	1.5–11	11
	14	6.98	26.8	2–9	9
	17	8.30	36.3	4–39	39
IV S^{35} Plasma	15	7.54	30.8	7–14	14
	18	7.80	30.0	12–35	35

TABLE X

Published biosynthetic turn-over data, human plasma proteins

Author	Reference	Labeling isotope	Number subjects	Half-life ($t_{\frac{1}{2}}$) in days: Total protein	Albumin	$\alpha+\beta$ globulins	Cohn I+III fraction	β_1 lipoproteins	Fibrinogen	γ globulins
				Normal subjects						
London	30	Oral N^{15} Glycine	2	7, 9*	20*					18, 19*
Kinsell *et al.*	19	IV S^{35} dl-methionine	3	9.2†						
Madden and Gould	44	Oral S^{35} Yeast protein	3 (? normal)						4.3, 5.0, 7.0	
Armstrong *et al.*	45, 48	Oral S^{35} Yeast protein	5		26–48	6–17				19–60
Niklas and Poliwoda	64	IV S^{35} l-methionine	1	22*‡	60					
Niklas and Poliwoda	64	Oral S^{35} l-methionine	8	23–41*‡ median 32						
			1		55					
Volwiler *et al.*	This paper	Oral S^{35} Cystine	7	14–39*‡ median 23.5	23–44 median 27.4		6.2, 7.8	3–7 median 6.1	5.6–8.0 median 6.7	48, 49, 85§
Volwiler *et al.*	This paper	IV S^{35} Normal plasma	3	13, 24*‡	17, 22, 26			2.9, 3.0, 3.8	3.4, 3.7, 4.2	20, 25, 35
				Cirrhosis						
London	30	Oral N^{15} Glycine	1		<20*					18*
Volwiler *et al.*	This paper	Oral S^{35} Cystine	5	18–36*‡ median 24.4	22, 25, 63		8, 10, 11	10	10.7, 10.9	31§
Volwiler *et al.*	This paper	IV S^{35} Normal plasma	3	30, 31*‡	23, 25, 26			3.4, 5.0, 6.1	4.0, 4.7, 5.2	40, 51

* Longer if only points after completion of mixing phase are considered.

† This figure was obtained by Tarver (31) by replotting the original data. Our replot of Kinsell's published data indicates a much longer half-time if only points after four days following isotope administration are considered (that is, after completion of mixing phase).

‡ s = serum.

§ Subjects followed longer than 20 days.

longer turn-over time for beta-1 lipoproteins in cirrhosis as compared with the normal state.

Half-lives obtained for serum total protein were considerably longer than reported for plasma total protein by others (Tables IX and X). The elimination through clotting of the rapidly turning-over fibrinogen component does not explain fully this discrepancy. The chief explanation is that these early reports included the usual two- to four-day mixing period in the calculation of half-life. The vast heterogeneity of molecular species and multiple differences between normal and abnormal subjects in specific plasma proteins does not invite comparison between normal and cirrhotic subjects for this determination.

The isolated gamma globulin fractions were composed of a continuous spectrum of gamma globulins (35). The same protein species were perhaps not present in our normal and cirrhotic subjects, and increases of total gamma globulin in the various cirrhotic patients could have represented different qualitative as well as quantitative

changes in these species. Therefore, as with total serum proteins, there is very limited value in comparing the half-life values between normal and cirrhotic subjects. For reasons mentioned, only gamma globulin values obtained following transfusion of donor-labeled plasma are considered significant (Table VIII).

DISCUSSION

The organic sulfur pool

Radioactive sulfur introduced in the form of l-cystine or tagged plasma protein containing S^{35}-labeled cystine may be expected to follow a distribution in the body's organic sulfur pool according to the pathways shown in Figure 6. This diagram points out the possible advantage of labeling less of the total pool by using tagged l-cystine as compared to the total labeling which would result from introducing S^{35} in l-methionine.

Basic general assumptions

Three assumptions are made in interpreting these turn-over data: 1) *All of the radioactive sulfur in the isolated protein fractions is tightly incorporated into the protein molecules as l-cystine or l-cysteine by peptide linkage.* Evidence that circulating S^{35}, within a few hours following its introduction into the body as cystine or methionine, exists firmly chemically-bound in plasma proteins has been previously furnished by both Tarver and Reinhardt (37) and Lee, Anderson, Miller, and Williams (38); mercaptoethanol treatment of the total serum proteins of one subject, according to the methods described by Lee, Anderson, Miller, and Williams (38) and by Peterson and Greenberg (39), did not disclose appreciable loose attachment of extra S^{35}-labeled cystine to circulating protein by disulfide linkage (Graph A, Figure 2). It has been generally assumed that all sulfur present in plasma protein molecules exists in the form of cystine, cysteine, or methionine residues attached by peptide bonds. However, other types of firm binding are possible: Studies *in vitro* by Schöberl (40) have shown that sulfur can be introduced into proteins by attachment of a sulfhydryl group onto the ϵ-amino nitrogen of lysine through action of polythioglycolide; and recently, the presence of a firm sulfate radical in bovine fibrinogen has been identified by Bettelheim (41); 2) *The rate of disappearance of the sulfur label reflects the rate of disintegration of the whole plasma protein molecule.* Certainly the manner in which plasma proteins are catabolized is at present unknown. Evidence indicating that, while circulating, certain plasma proteins do not exchange their amino acid residues has been presented by Humphrey and McFarlane (42); 3) *The rates of synthesis and degradation are equal for a plasma protein.* This seems probable since all subjects were in a relatively "steady state," both prior to and during the period of observations, with unchanging concentrations of serum proteins, regular daily activities, relatively constant high protein diet, and stationary state of the liver disease if present.

Errors of half-life determination

It is difficult to explain adequately the wide variation in apparent half-lives within specific fractions as obtained with S^{35}. Aside from normal expectation of biologic variation, errors and limitations of the laboratory techniques must be scrutinized. Random errors of this type could result in the scattering of small groups of plotted points so as to falsely orient the isotope die-away curve. The likely magnitude of this error was found small, there being little possible variation in choice as to slope of the isotope curve. An additional unassessed influence is the variation between individual subjects in the homogeneity of any specific plasma protein fraction isolated. Even relatively small amounts of protein contaminant having a markedly different half-life could significantly alter the values for the major component. Differences in physical and chemical properties, and concentrations of the various plasma protein components in both normal and abnormal sera might be expected to influence the reproducibility of the fractionation procedures employed. In a single subject, ultracentrifugal and electrophoretic analyses of a fraction isolated at different intervals following isotope administration showed reproducible though not always absolute homogeneity. However, even if a fraction were homogeneous by these criteria there is still the possibility of different molecular species being present in the protein.

Following oral administration of S^{35}-labeled

cystine, an error of unknown degree in prolonging the apparent half-life of plasma proteins may result from reutilization of isotope contributed to the body's organic sulfur pool by the catabolism of these and other labeled body proteins. The experiments in transfusing normal donor-labeled S^{35}-labeled plasma were carried out to appraise this problem. Even here, some reutilization by the more slowly turning-over fractions of the isotope contributed by the rapidly catabolized plasma proteins seems possible. This would seem particularly likely if the principal cellular site of destruction of the more rapid turning-over components were identical with the site of synthesis of other fractions. Slightly shorter half-times for fractions studied after plasma transfusion as compared with half-times of corresponding fractions measured after administering cystine suggests that isotope reutilization may produce error in prolonging the apparent rate of loss of isotope; a further accumulation of data has therefore reversed our previous opinion of this problem (43). Variation between subjects in degrees of isotope reutilization could account for differences in various half-lives. None of our subjects received during study supplements of non-labeled sulfur amino acids beyond these amounts contained in the usual high-protein diet. Though, logically, a further such flooding of the organic sulfur pool with non-labeled compounds should cause appreciable dilution of the labeled amino acids and thereby limit reutilization of isotope during synthesis of plasma proteins, conclusive data on this point have not yet been published. Indeed, the small amount of negative data from experiments in which supplementary non-labeled l-methionine or l-cystine was administered (presented by Madden and Gould [44], by Armstrong, McLeod, Wolter, and Kukral [45], and obtained in two pilot experiments on dogs by us) raises considerable doubt as to whether such influence would be of any real importance in these determinations.

Comparison of S^{35} with I^{131} labeling

Using S^{35} labeling, the turn-over time obtained for albumin by either of the two biosynthetic methods was much longer in two of the three subjects compared than determined by injecting I^{131}-labeled albumin [23] (Table IV). Our half-times for iodinated albumin lie within the lower portion of the range found by Berson, Yalow, Schreiber, and Post (34) employing iodinating albumin techniques considered most satisfactory. In our experiments also, urinary I^{131} excretion rates agreed in general with plasma I^{131} disappearance, and reflected a changing exponential rate of I^{131} discard (Figure 7). As noted by Berson, Yalow, Schreiber, and Post, the isotope disappearance curves of our I^{131}-labeled albumin also showed in each subject the presence of components rapidly losing the iodine label. The plotted data for plasma eventually assumed a steady exponential rate of isotope loss; [24] this slowest component has been selected as the half-time for iodinated albumin herein reported (Table IV). Even greater differences have been noted between the half-times of S^{35} and I^{131}-labeled gamma globulins; the S^{35} turn-over values observed in our laboratory and those obtained by Armstrong and co-workers (45, 48) are considerably longer than reported (48–53) for I^{131}-labeled human gamma globulin. Three explanations for this discrepancy in exponential rates are possible: (a) Proteins may be partially denatured by the iodinating techniques employed; (b) Introduction of the iodine atom may alter physical and biologic properties of native protein; and (c) Reutilization of S^{35} following biosynthetic labeling causes lengthening of apparent turn-over time.

Considerable evidence has accumulated to indicate that *in vitro* iodination of proteins may have a variable effect upon physical characteristics and biologic properties with considerable shortening of life-span: Berson, Yalow, Schreiber, and Post (34) have shown that turn-over times for iodinated human albumin may vary according to the

[23] Margen and Tarver (46, 47) have also noted in the same normal subject shorter turn-over times for I^{131}-labeled albumin than obtained for S^{35}-labeled albumin; S^{35} turn-over was measured both after administration of S^{35}-labeled amino acids and following intravenous injection of donor-labeled S^{35} albumin. Slowest components of the iodinated albumin preparations gave half-time values of 17 to 25 days, as compared with 24 to 30 days for intravenously administered S^{35}-labeled albumin and 36 to 60 + days for S^{35}-labeled albumin following intravenous administration of S^{35}-labeled amino acids.

[24] Following the eighth day for subject 9, but after the twentieth day in subjects 10 and 18.

particular chemical iodinating technique employed. Berson's data and ours demonstrate the non-uniform rate of I^{131} disappearance usually observed following the injection of iodinated albumin. Gabrieli, Goulian, Kinersly, and Collet (54) have demonstrated electrophoretic non-homogeneity of certain iodinated albumins. Iodination of growth hormone (55) or of insulin (56) may alter physiologic activity of these proteins; in addition, the possible influence of radiation denaturation from I^{131} has been mentioned (57). Whether intravenous transfer of biosynthetically labeled plasma proteins will allow more accurate determination of biologic life span than will present-day *in vitro* chemical labeling techniques has not yet been determined.

Interpretation of fibrinogen, beta-1 lipoprotein and gamma globulin turn-over curves

The interpretation of the turn-over curves for β_1 lipoprotein and fibrinogen is particularly difficult and uncertain. At least four explanations for the changing rate of isotope discard must be considered: (a) *Re-introduction of isotope label.* Since these appear to be very rapidly turning-over proteins with respect to the S^{35} label, reintroduction of discarded isotope could occur to a greater degree than found in the more slowly metabolized plasma proteins. The degree of reutilization might be much greater than in other fractions if a principal site of catabolism of these or other highly-labeled proteins were identical with the locus of synthesis of fibrinogen and beta-1 lipoprotein. (b) *Non-homogeneity of protein fraction, containing components of differing turn-over rates.* Presumably this beta-1 lipoprotein fraction is not a homogeneous molecular species (11, 58), while the fibrinogen fractions appraised were non-homogeneous electrophoretically and by clottability. (c) *Localized variations in metabolic rates.* Different metabolic sites may have different rates of synthesis and degradation of a specific protein or exchange varying quantities of cystine residues and at different positions in the protein molecule. In the rat, the data of Miller, Bale, and Bly (59, 60) indicate principal hepatic origin of plasma proteins other than gamma globulin. (d) *Prolonged and inefficient mixing of labeled proteins among various body fluid compartments.* Evidence from various sources is accumulating to indicate that the different plasma protein-containing fluid compartments of the body vary in their efficiency and speed of equilibration with circulating plasma. In addition, the possibility exists that different plasma proteins do not equilibrate among these fluid pools at the same rate.

Although these various influences cannot be individually assessed, the error from reintroduction of isotope appears important and increases proportionately with time; therefore, the slope of the straight-line early portion of Phase III (4 to 12-day period) of the curve of decline in specific activity has been chosen as the apparent half-time to be reported herein for each of these two fractions (Tables V and VI).

For the purposes of describing the general character of the isotope die-away curves for fibrinogen and beta-1 lipoprotein samples, these curves have been analyzed graphically and found to be resolvable into a 2-component exponential system (Figure 4). This type of mathematical analysis has become well established for describing certain varieties of biological systems as studied with isotopic tracers (62, 63). In the present experiments, the biochemical system is so complex that the meaning of such derived constants is unknown. Presumably each derived constant is actually composed of a family of varying exponential rates describing the various types of isotope transfer within the body's organic sulfur pool. Neither of these constants can be assumed to represent the exact turn-over rate of a protein. However, in the experiments in which transfused labeled plasma was administered, reutilization of isotope appears to have minimal influence upon the complexity of this system, and it is therefore suggested that the fast derived component may approach the true rate of discard of S^{35} from the principal protein species. Also, in the plasma transfer experiments, the slow derived constant may reflect chiefly the rate of turn-over of a contaminating slower catabolizing protein.

In considering the isotope curves for gamma globulins of subjects receiving oral S^{35}-labeled cystine, the strikingly long delay after maximal labeling before appreciable loss of specific activity occurred suggests strong influence of either selective reutilization of isotope or delay in distribution into plasma of newly-formed, highly-labeled

gamma globulin components. Therefore, for this fraction, values obtained after transfusion of labeled plasma seem far more significant.

Comparison of turn-over data between normal and cirrhotic subjects

It is of particular interest to note that the total mass of circulating plasma albumin in the patients with cirrhosis having low albumin concentrations was within the range of values found for normal subjects (Table IV). Presumably this reflects dilution of circulating albumin by increase in plasma volume related, in part, to expansion of collateral portal venous circulation. Of greater accuracy in such comparison would have been actual determination of Total Exchangeable Albumin; in edema-free subjects (normals and cirrhotics), the total amount of extravascular albumin was found by Berson, Yalow, Schreiber, and Post (34) to be surprisingly constant among various individuals, and averaged 60 per cent of the body's total albumin mass.

Our results with S^{35} for albumin turn-over suggest that patients with advanced portal cirrhosis having low plasma albumin concentrations may have a normal rate of albumin synthesis and catabolism. In some, this is understandable inasmuch as the determined total circulating plasma albumin mass may actually lie within normal limits. In those subjects having smaller than normal albumin pools, it could be suggested that the damaged liver's albumin-synthesizing machinery was simply smaller and therefore its daily total production is less; to maintain constant size of the body's albumin pool, daily albumin catabolism would then also need to decrease proportionately.

The difficulty of determining if there are differences from the normal in life-span of various molecular species in advanced cirrhosis is increased by the apparent spread of half-time values of the various fractions in each group of subjects. Certainly, metabolically significant alterations of half-lives could exist in diseased states which would fall within such a wide spread of values for the normal, therefore remaining undetected. Only very gross discrepancies in biologic life span would be recognized under these conditions. The small number of biosynthetically-determined life spans of the various plasma proteins accumulated in normal subjects and in patients with cirrhosis (Table X) does not permit final statements of clear comparison.

SUMMARY

1. An attempt has been made to measure turnover times in normal and cirrhotic human subjects for total serum proteins, albumin, β_1 lipoprotein, fibrinogen, and gamma globulins, using oral S^{35} cystine and infused S^{35}-labeled normal plasma.

2. The plasma protein fractions analyzed were isolated by means of differential solubility, selective ion-binding, and ultracentrifugal flotation.

3. Accurate determination of exact rates of plasma protein synthesis and degradation have not been possible with these techniques. Mixing times, delays in return of protein or isotope to the circulating plasma, reutilization of isotope, and other numerous complexities of the biochemical system are such that the specific activity die-away curves obtained cannot be accepted as representing the true degradation rate of the protein fraction at any time in the experiments.

4. Transfused labeled plasma protein yielded shorter apparent turnover times than were obtained following oral administration of the labeled amino acid. This supports the conclusion that reutilization of isotope liberated from the catabolism of labeled body proteins does introduce an appreciable error of apparent lengthening of the $t\frac{1}{2}$ times. Since this error would seem minimal for those experiments in which intravenous transfer of donor-labeled plasma protein was accomplished, it is believed that the $t\frac{1}{2}$ values obtained by this means may approach the true turn-over rates of the various isolated plasma protein fractions.

5. Fibrinogen and β_1 lipoprotein appear to be very much more rapid in turn-over with respect to the sulfur label than do albumin, gamma globulins or total serum proteins. After transfusing labeled plasma, apparent half-life values in the normal subject for fibrinogen and β_1 lipoprotein averaged 3.5 days as compared with a median of 21.5 days for albumin and 25 days for gamma globulins.

6. Comparison in the same subject of "turnover" of chemically iodinated albumin with "turn-

over" of biosynthetically sulfur-labeled albumin demonstrated a variety of populations of iodinated albumin molecules having differing rates of catabolism; this suggested partial denaturation of albumin by the iodinating method. Those albumin components slowest in rate of discard of I^{131} gave an apparent half-life slightly shorter than was obtained with S^{35} labeling.

7. Variation in apparent half-life rendered comparison difficult between normal and cirrhotic subjects. β_1 lipoproteins appeared to have a slightly longer biologic life-span in the cirrhotic, whereas no gross differences were noted for albumin, fibrinogen or total serum proteins. Data on gamma globulins were insufficient for comparison.

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APPENDIX

Details of patients with cirrhosis

Case 11, E.N.: Male Age 69, KCH 68870, Studied June, 1951. Epidemic viral hepatitis 1925. Non-alcoholic. Admitted KCH May, 1950 for massive upper gastro-intestinal hemorrhage. Initially, clinical criteria of cirrhosis absent. Surgical exploration revealed cirrhosis, portal hypertension, esophageal varices; splenic artery ligated. Hemorrhage recurred followed by ascites. After improvement, direct porta-caval shunt accomplished September, 1950; thereafter continuously hospitalized in chronic disease unit, where study was conducted.

Case 12, P.V.: Male, Age 58, KCH 118138, Studied June, 1951. Moderate wine intake for many years. In 1949 noted abdominal swelling. First KCH admission June, 1950 with spider angiomata, marked hepatic dysfunction. Needle liver biopsy March, 1951: advanced portal cirrhosis. Second KCH admission May, 1951 for anorexia and jaundice; following convalescence in chronic disease unit, transferred to Metabolic Research Ward.

Case 13, F.E.: Male, Age 51, KCH 274770, Studied January, 1952. Bar-tender with moderate, steady alcohol intake for many years. Jaundice in October, 1950. In December, 1950, onset of ascites requiring two paracenteses. First KCH admission February, 1951 with ascites, right hydrothorax, peripheral edema, hepatomegaly. Following interval care in Out-Patient Gastrointestinal Clinic, readmitted December, 1951 with ascites, right hydrothorax, peripheral edema, hepatomegaly, splenomegaly. After diuresis, transferred to Metabolic Research Ward for study.

Case 14, H.H.: Male, Age 48, KCH 234187, Studied April, 1953. Prolonged, large intake of alcohol, especially after 1943. First jaundiced 1937. Hospitalized in Minneapolis, Minn. 1946 for jaundice. In 1949, prolonged hospitalization in University Hospitals, Iowa City, Iowa with findings of jaundice, spider angiomata, peripheral edema, hepatomegaly. Liver biopsy: portal cirrhosis. First KCH admission November, 1952 with findings of spider angiomata, hepatomegaly, splenomegaly, ascites; following prolonged convalescence in chronic disease unit, transferred to Metabolic Research Ward.

Case 15, A.K.: Male, Age 52, KCH 303167, Studied June, 1953. Large alcoholic consumption for 30 years. In 1952 noted gradual abdominal swelling. Admitted KCH March, 1953 with jaundice, ascites, right hydrothorax, peripheral edema, spider angiomata, hepatomegaly, splenomegaly. After convalescence in chronic disease unit, transferred to Metabolic Research Ward.

Case 16, R.L.: Female, Age 40, KCH 301438, Studied October, 1953. Large alcohol intake for 10 years. Hospitalized 1952 for nausea, anorexia, hepatomegaly. First admitted KCH January 1953 with delirium tremens; findings included menorrhagia, jaundice, hepatomegaly, spider angiomata, ascites. Readmitted KCH September, 1953 with ascites, edema, hepatomegaly, splenomegaly. Convalescence continued in chronic disease unit, from which patient was transferred to Metabolic Research Ward.

Case 17, W.H.: Male, Age 51, KCH 223754, Studied January, 1954. Large alcohol intake for 15 years. Recurring jaundice 8 years. Five KCH admissions between 1948 and 1953 for jaundice, delirium tremens, edema, hepatic coma. Continuously hospitalized after September, 1953 with transfer from chronic disease unit to Metabolic Research Ward.

Case 18, C.S.: Male, Age 40, KCH 263080, Studied May, 1954. Merchant seaman with steady alcohol intake since age 19, increased after age 27. First jaundice 1948. Entered Marine Hospital, Seattle, 1949 with jaundice; diagnosis of portal cirrhosis made. First KCH admission 1951 with jaundice, ascites, peripheral edema, and hepatomegaly. Needle liver biopsy on admission: subacute fatty cirrhosis. Repeat biopsy four weeks later: satisfactory parenchymal repair, considerable fibrosis. Readmitted to KCH January, 1954, with recurrent ascites, jaundice, upper gastro-intestinal hemorrhage of

undetermined source; following prolonged convalescence in chronic disease unit, transferred to Metabolic Research Ward.

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PROTEIN–LIPID RELATIONSHIPS IN HUMAN PLASMA: IN BILIARY CIRRHOSIS, OBSTRUCTIVE JAUNDICE, AND ACUTE HEPATITIS [1]

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In 1862, Austin Flint (1) found that cholesterol accumulated in the blood in obstructive jaundice. Fifty years later Widal, Weill, and Laudat (2) pointed out that the hypercholesterolemia of obstructive jaundice is due in large measure to increase in serum free cholesterol in contradistinction to that of nephrosis, where both free and esterified cholesterol are greater than normal. More recently (3–5) it has been shown that plasma phospholipid increases even more than cholesterol, so that the ratio of total cholesterol to phospholipid is appreciably less than normal.

The serum lipoproteins have also been measured in patients with liver disease. Pierce and Gofman (6) examined the sera of thirty-two patients with cirrhosis and found levels of the S_f 10–20 class slightly higher than normal. They also studied forty-eight cases of acute hepatitis (7) and found elevations of the S_f 0–100 components with the largest increase in the S_f 12–20 class. The increased concentrations correlated most closely with the presence of jaundice and tended to decrease when the jaundice subsided. McGinley, Jones, and Gofman (8) found a huge increase in the S_f 6 and S_f 8 groups, with varying increase in S_f 10–17 in five cases of biliary cirrhosis. By starch zone electrophoresis, Kunkel and Slater (9) observed that in biliary cirrhosis there was a high peak consisting predominantly of phospholipid which had the same relative mobility as normal beta lipoproteins. The concentration of alpha lipoproteins was extremely low. Snavely, Goldwater, Randolph, and Unglaub (10) by ultracentrifugation of whole serum from patients with acute hepatitis found an increase in light aggregates rich in neutral fat and poor in ester cholesterol.

In this present study, plasma proteins in patients with liver diseases in which biliary obstruction is a prominent or suspected feature were fractionated by Cohn's method number 10 and the fractions analyzed for protein and lipid constituents. In some of the patients, and especially in those with acute hepatitis, it was possible to analyze the plasma serially during the disappearance of jaundice and during the various phases of recovery from the disease.

SUBJECTS

The cases studied were classified as follows.

	Number of cases
Biliary cirrhosis	
Primary	7
Secondary	5
Bile duct obstruction	12
Acute hepatitis	30
Portal cirrhosis	36

The diagnosis of primary biliary cirrhosis was made on the basis of the clinical picture. In six of the seven patients (Table IIA), exploratory laparotomy revealed an appearance of the liver indicating biliary cirrhosis. There was no evidence of extrahepatic biliary obstruction. In one patient (Yaz), laparotomy was not performed, but needle biopsy of the liver yielded a specimen regarded as histologically typical of biliary cirrhosis. The duration of illness varied from six months in Pey and Ros, to eight years in Whe and Dor. In Pey, xanthomata were not present (perhaps because of the short duration of the disease). All of the other patients had xanthelasma, and with the exception of Whe had developed xanthomata in other areas. All of the patients had large livers and spleens. All of them were intensely jaundiced and had high concentrations of alkaline phosphatase at the time of plasma fractionation.

The diagnosis of secondary biliary cirrhosis was made in five patients with chronic jaundice in whom laparotomy

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and biopsy of the liver presented a picture compatible with biliary cirrhosis but in whom there was also evidence of extrahepatic biliary obstruction (Table IIB). In Kur and Fit the common bile duct had been damaged by previous surgery. Sto had a large congenital pancreatic cyst. Although no common duct stone was found in Drm and Bro, they were classified as secondary biliary cirrhosis because of chronic cholecystitis and the presence of stones in the gall-bladder. Duration of symptoms was from one and one-quarter years in Drm to eleven years in Fit and Sto. Drm, Fit, and Bro had xanthelasma but no evidence of xanthomatosis elsewhere. The others displayed no cutaneous lipid deposits. Two of the five patients (Drm and Fit) were not intensely jaundiced at the time of fractionation. All had serum alkaline phosphatase concentrations greater than thirty Bodansky units.

The group designated as bile duct obstruction (Table III) consisted of six patients with carcinoma of the pancreas, three with common duct stone, two with metastatic carcinoma of the liver, and one who had both hemachromatosis and primary liver cell carcinoma. The duration of jaundice varied from one to five weeks. All patients were jaundiced at the time that they were studied; in Kau and Smi subsequent studies were made following the relief of obstruction. In these patients, serum alkaline phosphatase concentration was elevated, and in Kau and Smi decreased along with serum bilirubin concentration.

Patients with acute hepatitis (Table IV) were not differentiated as to etiology. Eight of the thirty had histories of blood transfusions; none of them had a history of exposure to toxic substances. For convenience, the date of onset of the disease was arbitrarily defined as the day on which jaundice or dark urine was first recognized. Since, however, many of the patients had suffered vague symptoms for some days preceding the icterus, it was impossible to determine the duration of the illness with accuracy. It was thought that, in eleven, studies were initiated during the first week; in five, during the second; in twelve, during the third and fourth; and in two, still later in the course of the illness. Those observed during the first week after onset probably represent a random sample of cases of hepatitis admitted to the hospital during the period. Those in whom observations were initiated at a later time usually had persistent icterus. All of the patients with jaundice had also elevated levels of serum alkaline phosphatase and abnormal cephalin flocculation and thymol turbidity. These abnormalities disappeared as the concentration of bilirubin decreased. Bat and Dre died within a week of onset, and autopsies showed acute massive necrosis of the liver. Saz and Ols had less fulminating courses and died after day 23 and 83, respectively. All the other patients recovered.

The group of patients with portal cirrhosis (Table V) included thirty with progressive hepatic insufficiency, peripheral edema, and ascites. All but three of them offered histories of large alcohol intake. The group also included one patient with hemochromatosis and five young women who gave no history of alcoholism but who suffered from acne, amenorrhea, recurrent attacks of fever, jaundice, and other features of the syndrome described by Bongiovanni and Eisenmenger (11). Several of the patients with alcoholic cirrhosis had episodes suggestive of superimposed obstructive jaundice with marked elevation of serum bilirubin and clay-colored stools. This was apparent in Rob on 2/20/52 and in Guz on 9/16/53. Sko had a hemolytic anemia with high indirect bilirubin and greater than normal excretion of urobilinogen in the stool.

METHODS

Cohn's method number 10 was used for the chemical fractionation of the plasma proteins. Three fractions were isolated: IV + V + VI, II, and I + III. Plasma and all fractions were analyzed for protein and for cholesterol by the Bloor method. In some of the cases free and total cholesterol, phospholipid, and total lipid carbon were determined in unfractionated plasma and in Fractions IV + V + VI and I + III. Details of the procedure of the fractionation of proteins and of the analytic methods have been previously described (12). Free and total cholesterol were determined by the method of Sperry and Schoenheimer as modified by Sperry and Webb (13). With very dilute samples it was necessary to concentrate the extracts by evaporation of solvent.

Paper electrophoresis was carried out by the method described in detail in a previous publication (14).

Ultracentrifugation was carried out in a Spinco model L preparative ultracentrifuge. The solvent density of the serum was raised to 1.063 by adding to 4 ml. of serum 4 ml. of NaCl solution containing 181.7 gm. per liter (15). Tubes were inverted and then centrifuged at 80,000 times gravity for thirteen hours at 15 to 16° C. The top layer was removed, using a hypodermic syringe and curved needle. The top and bottom layers were analyzed for lipid and subjected to electrophoresis on paper.

RESULTS

In Tables II to V the results of cholesterol and phospholipid analyses of plasma and of the protein fractions have been assembled together with values for total serum bilirubin and the ratios of free to total cholesterol on the plasma. Observations on the Sperry-Schoenheimer (13) determinations and the free to total ratios of the fractions are presented separately in Table VI.

In order to facilitate comparison, normal values and ranges for two age groups have been listed in Table 1. For cholesterol by the Sperry-Schoenheimer method, normal values are available only for the younger group. In the older age group the values would probably be altered in a direction similar to that of the cholesterol values by the Bloor method (12).

TABLE I

Normal values of concentration and distribution of protein, cholesterol, and phospholipids in plasma and fractions of normal young men and women

	Young men and women aged 18–35	Older men and women aged 45–65
Cholesterol (Bloor)		
Plasma (mg. per 100 ml.)	199 (126–276)	261 (171–354)
Fraction IV+V+VI (mg. per 100 ml.)	58 (39–93)	54 (32–80)
Fraction I+III (mg. per 100 ml.)	134 (47–228)	187 (95–294)
Phospholipid		
Plasma (mg. per 100 ml.)	228 (148–287)	270 (202–327)
Fraction IV+V+VI (mg. per 100 ml.)	113 (73–164)	102 (75–140)
Fraction I+III (mg. per 100 ml.)	106 (46–157)	155 (74–244)
Cholesterol-phospholipid ratio		
Plasma	0.87 (0.62–1.20)	1.00 (0.79–1.18)
Fraction IV+V+VI	0.51 (0.32–0.64)	0.51 (0.35–0.61)
Fraction I+III	1.26 (0.71–1.56)	1.38 (1.12–1.49)
Ester cholesterol (Sperry-Schoenheimer)		
Plasma (mg. per 100 ml.)	123 (89–157)	
Fraction IV+V+VI (mg. per 100 ml.)	33 (21–51)	
Fraction I+III (mg. per 100 ml.)	81 (52–106)	
Free cholesterol		
Plasma (mg. per 100 ml.)	49 (42–64)	
Fraction IV+V+VI (mg. per 100 ml.)	11 (7–15)	
Fraction I+III (mg. per 100 ml.)	39 (27–50)	
Free/Total cholesterol		
Plasma	0.29 (0.22–0.35)	
Fraction IV+V+VI	0.24 (0.20–0.28)	
Fraction I+III	0.33 (0.27–0.48)	

Primary biliary cirrhosis

In Table IIA the results obtained in the eight cases of primary biliary cirrhosis have been assembled. Because the studies were made at various stages of the disease, each case is considered individually and averages are not determined. In the Table, the cases are arranged in the order of decreasing cholesterol concentration. In every case of the group the total cholesterol is greater than normal, although it tends to diminish in those of longer duration. The increased cholesterol tends to accumulate chiefly in Fraction IV + V + VI where in three of the cases it constitutes 70 per cent or more of the total concentration. In all of the patients, the phospholipids of the plasma increased more than the cholesterol with resultant marked lowering of the cholesterol-phospholipid ratios. This is almost entirely attributable to a disproportionate accumulation of phospholipids in Fraction I + III where the cholesterol-phospholipid ratios are grossly lower than normal and frequently approximate those of Fraction IV + V +

VI where phospholipids increased proportionately to the cholesterol and where the cholesterol-phospholipid ratios deviated little from the normal average of approximately 0.5.

Analysis of the plasma by the Sperry-Schoenheimer method shows that the increment in plasma cholesterol in all of the patients is due to accumulation of free cholesterol with a resultant increase in the ratio of free to total cholesterol. Even in Dor, however, where there is no increase in total plasma cholesterol, the free to total ratio is also markedly elevated. Analysis of several of the fractions (see Table VI) indicates that almost all of the cholesterol in Fraction IV + V + VI is free, while Fraction I + III contains essentially all of the plasma esterified cholesterol in addition to free cholesterol.

In Table IIB are the data from the five cases of secondary biliary cirrhosis. The concentration of plasma cholesterol exceeds the limits of normal but is increased less than in the cases of primary biliary cirrhosis. With progressive hepatic failure the concentration tends to fall. The pattern of distribution of cholesterol is similar to that in primary biliary cirrhosis. In all patients, concentration of bilirubin is markedly elevated, the cholesterol-phospholipid ratios are considerably reduced, and the free to total cholesterol ratio markedly increased both in the plasma and in Fraction I + III. In Sto, as the jaundice increases the cholesterol-phospholipid ratios in plasma and Fraction I + III decrease, and the ratio of free to total cholesterol rises (see Table VI).

Bile duct obstruction

In Table III, the data on the patients with bile duct obstruction have been assembled. Patients were selected on the basis of considerable constant or increasing jaundice. Cases in which jaundice was decreasing were not included, although in two of the cases (Kau and Smi) measurements made after jaundice had begun to subside are included. In four of the patients, cholesterol in the plasma as well as in the two lipid-containing fractions is elevated. All of the patients in this group have low cholesterol-phospholipid ratios in the plasma and in Fraction I + III. In nine of the twelve cases in which cholesterol-phospholipid ratios and free to total cholesterol ratios are extremely abnormal, history indicates that the obstruction has been of less than six weeks' duration. These patients with bile duct obstruction of relatively short duration and presumably normal livers demonstrate most clearly the effects of obstructive jaundice on plasma cholesterol-phospholipid ratios and free to total ratios. Restoration of normal lipid interrelationships after relief of obstruction is indicated in Smi.

Acute hepatitis

In Table IV, the data on the thirty cases of acute hepatitis have been assembled. The patients are listed in order of time elapsed between the onset (the day when jaundice or bilirubinemia was first noted) and the first analysis of the plasma.

The deviations in lipid concentration and distribution vary both with the duration of the hepatitis and the degree of icterus. In severely jaundiced cases studied during the first week following onset, the level of total plasma cholesterol is appreciably reduced, the concentration of phospholipids is not proportionately reduced, with the result that there is a marked lowering in the cholesterol-phospholipid ratio both in the plasma and in Fraction I + III. In these cases there is also a marked reduction in esterified cholesterol, with increase in the ratio of plasma free to total cholesterol. This is usually apparent also in Fraction I + III but is most marked in Fraction IV + V + VI, where almost none of the cholesterol is esterified (Table VI). In patients whose total cholesterol is less than normal, the percentage in Fraction IV + V + VI tends to be low. In the occasional patient with high concentration of total cholesterol there is an increase in the cholesterol in Fraction IV + V + VI such as is seen in biliary cirrhosis.

These striking deviations in concentration and distribution of lipids tend to disappear gradually with recovery from the disease and particularly with the subsidence of icterus. They may disappear entirely before clinical convalescence is complete. Among the nineteen cases whose study was begun more than a week after the onset, there were seven whose bilirubin concentration was less than 2 mg. per 100 ml. In these cases, low cholesterol-phospholipid ratios in the plasma and in Fraction I + III were not found. In patients fol-

TABLE II

Protein-lipid relationships in biliary cirrhosis

Name	Age	Sex	Duration of jaundice *years*	Date	Total serum bilirubin *mg./100 ml.*	Cholesterol				Phospholipid			Chol./P'lipid		
						Plasma *mg./100 ml.*	Fractions IV+V+VI *mg./100 ml.*	Fractions I+III *mg./100 ml.*	Free/Total cholesterol plasma	Plasma *mg./100 ml.*	Fractions IV+V+VI *mg./100 ml.*	Fractions I+III *mg./100 ml.*	Plasma	Fractions IV+V+VI	Fractions I+III
A. Primary biliary cirrhosis															
Pey	68	F	½	10–2–50	6.6	783	535	143	0.73	1,595	1,225	268	0.49	0.44	0.59
				1–19–53	10.2	955	595	220	0.65	1,695	1,168	283	0.56	0.51	0.79
				7–14–53	10.0	1,190	855	237	1.00	2,362	1,684	384	0.50	0.51	0.62
Ros	35	F	½	12–4–51	6.8	1,180	833	296	0.90	2,237	1,532	481	0.53	0.54	0.62
Bai	40	F	1½	2–3–53	21.0	866	591	270	0.92	2,058	1,461	447	0.42	0.40	0.60
				2–20–53	18.5	906	590	310		1,895	1,315	480	0.48	0.45	0.63
Kel	59	F	2½	10–21–53	4.4	639	285	321	0.76	996	487	429	0.64	0.58	0.75
Yaz	35	F	3	9–2–52	12.0	576	345	213	0.76	1,347	876	281	0.43	0.40	0.56
Whe	54	F	8	4–8–53	11.3	400	186	199	0.80	789	445	303	0.51	0.42	0.66
Dor	55	F	8	7–30–53	32.0	250	98	151	0.83	377	157	225	0.66	0.62	0.52
B. Secondary biliary cirrhosis															
Kur	68	F	3	11–6–50	7.8	410	170	199		708	338	213	0.58	0.50	0.93
				6–4–52	11.0	188	37	149	0.61	321	92	190	0.59	0.41	0.78
Drm	33	F	1¼	6–19–53	1.1	369	49	313	0.44	348	93	244	1.06	0.53	1.28
Fit	39	F	1½	11–6–50	1.9	354	126	178		472	251	142	0.75	0.50	1.25
Bro	53	F	2	8–3–53	13.6	328	124	197	0.81	547	262	249	0.60	0.47	0.79
Sto	11	F	7	11–1–50	6.0	296	43	202		342	117	176	0.87	0.37	1.08
				11–19–51	11.2	241	35	205	0.51	360	83	247	0.67	0.42	0.83
				12–29–52	24.9	109	20	87	0.77	220	41	176	0.49	0.49	0.49

TABLE III

Protein-lipid relationships in bile duct obstruction with constant or increasing jaundice

Name	Age	Sex	Duration of jaundice	Date	Total serum bilirubin *mg./100 ml.*	Cholesterol				Phospholipid			Chol./P'lipid		
						Plasma *mg./100 ml.*	Fractions IV+V+VI *mg./100 ml.*	Fractions I+III *mg./100 ml.*	Free/Total cholesterol plasma	Plasma *mg./100 ml.*	Fractions IV+V+VI *mg./100 ml.*	Fractions I+III *mg./100 ml.*	Plasma	Fractions IV+V+VI	Fractions I+III
Zik	55	F	2 wks.	9–7–51	20.8	1,032	815	189	0.92	2,575	1,890	145	0.40		
Nie	59	F	5 wks.	11–12–53	12.4	644	477	159	0.74	1,321	1,025	295	0.49	0.47	0.54
Kau	54	F	1 wk.	11–23–53	24.6	517	272	246	0.78	983	557	386	0.53	0.49	0.64
				12–16–53	2.6	317	58	251		342	111	231	0.93	0.44	1.20
Mee	54	F	1 wk.	10–6–50	13.0	435	160	215	0.65	765	379	343	0.57	0.42	0.63
Lei	64	F	4 dys.	11–24–53	10.2	324	68	248	0.48	444	149	274	0.73	0.46	0.91
Smi	68	M	2 wks.	2–26–53	5.7	284	95	184	0.61	517	242	238	0.55	0.39	0.77
				3–18–53	2.2	267	113	151	0.38	389	205	142	0.69	0.55	1.06
				7–27–53	1.0	240	40	194	0.47	305	92	191	0.79	0.44	1.02
Fri	63	M	3 wks.	3–10–52	11.4	232	29	200	0.38	325	84	220	0.71	0.34	0.90
Cam	81	M	2 wks.	2–17–53	8.9	206	30	173	0.35	300	91	205	0.69	0.33	0.84
Ros	75	M	1 wk.	7–9–51	18.1	212	59	140		424	192	201	0.50	0.31	0.70
Mor	64	M	2 wks.	7–20–53	21.0	197	31	166	0.80	339	62	277	0.58	0.49	0.60
Tie	64	M	3 wks.	1–6–53	6.4	169	35	132	0.43	306	115	186	0.55	0.31	0.71
Mas	51	M	4 wks.	2–2–53	22.8	110	36	72	0.96	178	55	127	0.62	0.65	0.57

TABLE IV

Protein-lipid relationships during the course of acute hepatitis

Name	Age	Sex	Date	Day	Total serum bilirubin mg. per 100 ml.	Cholesterol				Phospholipid			Chol./P'lipid		
						Plasma mg. per 100 ml.	Fractions		Free/Total cholesterol plasma	Plasma mg. per 100 ml.	Fractions		Plasma	Fractions	
							IV+V+VI mg. per 100 ml.	I+III mg. per 100 ml.			IV+V+VI mg. per 100 ml.	I+III mg. per 100 ml.		IV+V+VI	I+III
All	50	M	4/8/52	4	12.0	151	32.5	113.9	0.68	339	89.5	201.5	0.45	0.36	0.56
			4/21/52	17	19.2	119	9.0	109.8	0.83	356	34.8	258.5	0.33	0.26	0.42
			4/28/52	24	7.9	210	17.3	182.0	0.53	378	51.0	293.8	0.56	0.34	0.62
			5/20/52	46	1.6	224	32.0	189.8	....	266	....	....	0.84	....	....
			8/25/52	97	0.5	174	26.7	145.0	0.26	224	72.5	138.0	0.78	0.37	1.05
Bat	29	M	5/19/52	4	14.5	75	5.0	70.5	0.32	120	11.2	103.0	0.62	0.45	0.68
Bey			4/24/52	5	7.6	160	25.6	130.5	0.48	338	89.9	187.0	0.47	0.28	0.70
			5/7/52	18	1.7	177	42.9	125.6	0.29	274	120.7	127.1	0.65	0.36	0.99
			5/20/52	31	1.2	160	57.3	101.9	....	237	....	....	0.68	....	....
			7/23/52	97	1.2	162	39.1	118.7	0.23	242	105.5	106.5	0.67	0.37	1.12
Wol	70	M	2/11/52	6	20.6	225	98.4	127.5	0.84	493	213.5	244.0	0.46	0.46	0.52
			2/19/52	14	24.0	204	84.2	122.2	0.79	461	183.0	246.0	0.44	0.46	0.50
			2/27/52	22	6.5	269	28.1	241.5	0.34	307	73.0	211.0	0.88	0.38	1.14
			3/3/52	26	5.2	311	42.0	266.0	0.27	316	92.9	181.7	0.98	0.45	1.46
			3/25/52	48	2.3	254	58.0	195.0	0.31	289	120.1	148.0	0.88	0.48	1.31
			4/30/52	84	0.7	264	54.2	182.1	0.27	298	112.9	148.0	0.88	0.48	1.23
Dav	31	M	3/25/52	6	7.4	201	35.2	163.2	0.64	386	110.5	245.8	0.52	0.32	0.67
			3/31/52	12	17.5	180	57.7	125.8	0.81	404	142.2	248.8	0.45	0.40	0.50
			4/7/52	19	26.6	195	54.2	146.8	0.83	396	111.1	269.9	0.49	0.49	0.55
			4/15/52	27	35.9	206	45.6	171.3	0.85	402	80.9	292.0	0.51	0.56	0.59
			4/23/52	35	40.8	194	55.0	139.6	....	373	76.5	271.0	0.52	0.72	0.52
			4/29/52	41	26.0	209	26.0	183.2	0.70	415	86.4	325.0	0.50	0.31	0.56
			5/6/52	48	11.0	295	26.0	269.0	0.45	351	62.9	274.1	0.84	0.41	0.98
			5/20/52	62	3.8	332	41.4	276.5	0.31	372	106.9	230.0	0.89	0.39	1.20
			5/27/52	69	2.3	278	49.8	228.0	....	325	105.4	194.0	0.86	0.48	1.17
			6/3/52	76	2.0	340	60.5	276.0	0.26	345	115.3	205.0	0.99	0.52	0.80
Vom	27	F	9/11/51	6	5.6	126	32.1	90.4	....	232	85.5	132.8	0.54	0.35	0.68
			10/4/51	29	0.7	242	64.7	163.0	....	267	137.0	107.0	0.91	0.47	1.52
			11/28/51	84	0.4	248	84.5	152.9	....	293	139.0	113.0	0.85	0.61	1.35
			7/21/52	319	0.2	187	48.7	127.6	....	242	113.3	110.0	0.77	0.43	1.15
Man	41	M	2/17/53	96	3.6	137	17.8	112.2	0.46	266	68.8	196.0	0.52	0.26	0.57
			3/4/53	21	0.4	286	35.6	250.0	0.31	325	88.2	225.0	0.88	0.40	1.11
Dre	52	M	10/13/53	7	12.1	84	0	85.5	0.89	93	0	89.5	0.80	....	0.96
Stu	26	M	10/22/51	7	6.7	177	66.2	103.0	0.76	406	162.0	197.0	0.44	0.41	0.52
			11/1/51	17	2.4	220	37.8	175.5	0.32	253	84.2	147.7	0.87	0.45	1.19
			11/12/51	28	1.4	202	43.2	155.5	0.26	252	104.5	124.5	0.80	0.41	1.25

TABLE IV—*Continued*

Name	Age	Sex	Date	Day	Total serum bilirubin *mg. per 100 ml.*	Cholesterol				Phospholipid			Chol./P'lipid		
						Plasma *mg. per 100 ml.*	Fractions		Free/Total cholesterol plasma	Plasma *mg. per 100 ml.*	Fractions		Plasma	Fractions	
							IV+V+VI *mg. per 100 ml.*	I+III *mg. per 100 ml.*			IV+V+VI *mg. per 100 ml.*	I+III *mg. per 100 ml.*		IV+V+VI	I+III
			11/26/51	42	...	193	50.0	129.5	....	241	105.0	112.0	0.80	0.48	1.16
			11/27/51	43	1.0	208	53.4	154.2	....	259	108.0	125.0	0.80	0.49	1.23
			12/5/51	51	0.9	171	36.8	125.0	....	...	....	....	....	....	....
			12/11/51	57	0.6	214	41.2	166.8	....	...	....	....	....	....	....
			1/23/52	100	1.3	188	50.2	141.1	....	224	103.6	100.8	0.84	0.48	1.40
			7/7/52	265	1.1	232	60.4	171.1	0.23	232	104.6	127.8	1.00	0.58	1.34
Wee	24	F	4/30/52	7	3.8	152	26.0	119.1	0.70	326	63.7	208.1	0.47	0.41	0.57
			5/7/52	14	3.2	174	27.1	148.4	0.39	216	63.4	140.0	0.83	0.43	1.26
			5/28/52	35	0.7	169	45.2	124.0	0.20	212	95.0	99.8	0.80	0.48	1.24
			7/15/52	83	0.3	208	63.4	141.5	....	...	....	....	....	....	....
Chr	27	M	3/3/53	7	3.3	122	30.0	92.0	0.83	287	89.0	181.0	0.43	0.33	0.52
			3/10/53	14	1.5	178	25.2	151.0	....	250	77.6	168.0	0.71	0.35	0.90
			3/17/53	21	0.8	188	32.4	....	0.63	226	79.0	....	0.83	0.41	....
			3/23/53	27	1.0	194	45.5	142.0	0.29	246	101.0	136.0	0.79	0.45	1.04
			5/11/53	76	1.3	194	46.0	142.0	0.32	203	90.7	112.0	0.96	0.51	1.27
Atk	25	M	10/22/51	10	1.5	208	37.8	166.1	....	248	81.5	146.5	0.84	0.46	1.13
			11/1/51	20	0.8	252	67.9	164.1	0.33	281	122.0	125.0	0.90	0.56	1.31
			11/12/51	31	0.8	238	68.5	147.0	0.26	296	144.4	123.2	0.80	0.47	1.20
			11/21/51	40	0.6	206	72.2	132.8	....	265	133.0	106.0	0.78	0.54	1.25
			12/3/51	52	...	195	64.4	130.1	....	...	....	....	....	....	....
Don	55	M	3/2/53	10	4.0	187	24.5	163.0	....	246	73.2	164.5	0.76	0.34	0.99
			3/10/53	17	0.6	216	49.6	160.0	....	266	114.0	141.0	0.81	0.44	1.13
Lau	31	F	11/5/51	13	15.4	192	14.2	171.8	....	357	61.2	252.0	0.54	0.23	0.68
			11/13/51	21	2.0	266	51.0	210.0	0.27	309	110.0	170.0	0.86	0.46	1.17
			11/21/51	29	1.4	302	59.7	236.0	....	368	124.0	190.0	0.82	0.48	1.24
			7/23/52	273	0.5	249	46.4	195.8	....	281	104.5	150.4	0.89	0.44	1.30
Joh	48	F	5/27/52	13	20.8	147	25.1	122.7	0.81	332	68.6	240.9	0.44	0.37	0.51
			6/9/52	26	22.2	260	78.5	184.0	0.85	532	183.0	330.5	0.49	0.57	0.61
			7/3/52	50	3.4	316	62.4	248.0	0.25	384	144.0	213.0	0.82	0.43	1.16
			7/17/52	64	1.9	288	63.6	220.0	0.23	308	121.6	180.1	0.94	0.30	1.22
Bou	22	M	3/24/52	14	1.8	253	41.1	197.8	0.58	369	109.1	226.0	0.69	0.38	0.88
			4/1/52	22	0.8	234	36.4	198.4	....	260	86.4	150.9	0.90	0.42	1.32
			5/27/52	78	...	152	42.9	107.0	....	195	95.8	89.8	0.78	0.45	1.30
Cal	21	M	3/24/52	15	35.1	129	23.2	102.1	0.79	236	38.6	187.0	0.55	0.60	0.55
			5/8/52	30	4.9	189	36.2	147.0	0.23	230	87.6	127.5	0.82	0.41	1.15
			4/15/52	37	4.5	238	40.5	197.0	....	272	85.0	158.6	0.87	0.48	1.24
			5/6/52	58	1.7	216	34.3	173.0	0.29	221	73.5	127.9	0.98	0.47	1.35

TABLE IV—*Continued*

Name	Age	Sex	Date	Day	Total serum bilirubin *mg. per 100 ml.*	Cholesterol				Phospholipid			Chol./P'lipid		
							Fractions				Fractions			Fractions	
						Plasma *mg. per 100 ml.*	IV+V+VI *mg. per 100 ml.*	I+III *mg. per 100 ml.*	Free/Total cholesterol plasma	Plasma *mg. per 100 ml.*	IV+V+VI *mg. per 100 ml.*	I+III *mg. per 100 ml.*	Plasma	IV+V+VI	I+III
Saz	73	M	11/10/52	16	35.3	88	5.5	78.5	1.00	167	8.9	140.0	0.53	0.62	0.56
Ran	23	F	1/7/51	16	14.0	283	143.0	126.0	0.86	598	307.0	252.0	0.47	0.47	0.50
			11/15/51	24	9.4	328	114.9	209.0	0.58	503	229.0	226.0	0.65	0.50	0.92
			11/21/51	30	4.6	287	60.6	224.0	0.33	365	128.0	206.0	0.79	0.47	1.09
			11/28/51	37	5.6	312	48.7	247.0	....	388	121.3	237.0	0.80	0.40	1.04
			12/5/51	44	3.6	250	42.4	210.0	....	311	101.4	183.0	0.80	0.42	1.15
Ste	25	M	2/18/52	17	4.8	125	6.7	110.5	0.42	219	41.3	159.5	0.57	0.16	0.55
			2/27/52	26	1.7	171	31.1	....	....	219	81.1	....	0.78	0.38	0.90
			3/3/52	30	1.2	180	35.0	134.2	....	242	83.5	126.2	0.74	0.42	1.06
Sch	26	M	8/31/51	18	0.9	203	19.5	79.0	....	247	41.0	164.0	0.82	0.48	1.09
Cin	46	F	3/19/53	18	1.5	190	18.5	168.9	....	264	57.8	191.0	0.73	0.32	0.89
Gre	30	F	4/14/52	19	1.4	236	49.5	182.5	0.32	368	108.1	201.8	0.64	0.46	1.10
			4/28/52	33	0.7	261	43.1	209.0	....	292	93.9	176.0	0.89	0.46	1.19
Hoo	41	M	1/7/52	20	28.8	136	21.8	114.4	0.86	336	55.2	256.0	0.40	0.40	0.45
			1/17/52	30	30.8	176	42.2	139.0	....	...	....	....	....	....	....
			1/23/52	36	29.3	198	38.1	161.9	0.67	452	84.2	320.0	0.44	0.45	0.51
			1/28/52	41	9.2	198	17.3	179.0	0.43	273	....	214.0	0.73	....	0.84
			2/4/52	48	4.1	234	29.0	200.0	0.26	300	70.0	202.0	0.78	0.41	0.99
			2/14/52	58	3.9	184	25.0	152.1	0.26	221	59.5	149.3	0.83	0.42	1.02
			2/21/52	65	2.1	174	28.8	139.9	0.29	222	64.0	139.0	0.78	0.45	1.01
			3/4/52	76	1.2	178	31.9	....	0.26	...	....	....	....	....	....
			4/3/52	106	...	186	25.8	152.7	0.20	218	70.9	134.1	0.85	0.36	1.14
			4/22/52	155	...	179	27.4	149.0	0.34	228	66.0	143.9	0.78	0.42	1.04
			7/29/52	224	...	179	28.8	185.2	0.24	225	75.8	131.0	0.81	0.38	1.11
Doy	51	F	7/23/51	21	1.2	210	44.3	159.0	....	293	106.0	145.0	0.72	0.42	1.10
Roj	20	M	8/3/53	26	9.4	205	62.1	140.9	....	389	145.4	245.0	0.53	0.43	0.38
For	33	M	9/10/51	28	26.6	131	14.7	107.8	....	306	48.4	214.2	0.43	0.30	0.50
			12/3/51	112	...	190	39.2	138.2	....	215	94.4	114.0	0.91	0.42	1.21
Far	32	F	4/16/53	28	0.6	195	28.6	158.1	....	249	73.5	165.0	0.78	0.39	0.96
Oel	66	F	1/14/54	34	24.2	258	18.1	241.2	0.76	500	67.0	426.0	0.52	0.27	0.57
			1/21/54	41	10.0	298	33.2	255.0	....	426	80.9	332.0	0.70	0.41	0.77
Ols	51	M	8/12/52	49	32.0	129	22.2	102.0	....	260	50.0	207.0	0.49	0.57	0.52
			9/11/52	79	8.0	66	12.1	51.9	0.67	146	36.6	101.5	0.45	0.33	0.51

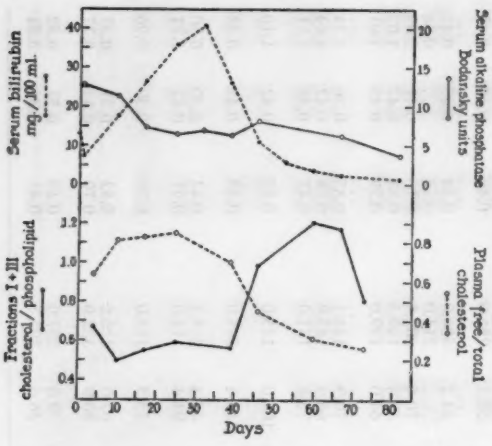

FIG. 1. RELATIONSHIP OF SEVERAL CHEMICAL TESTS DURING THE COURSE OF A TYPICAL CASE OF HEPATITIS

lowed serially from the acute phase through recovery, the cholesterol-phospholipid ratio in the plasma and in Fraction I + III was observed to rise simultaneously with the fall in serum bilirubin concentration and the ratio of free to total cholesterol. The return to normal in cholesterol and phospholipid relationships appears to be related to recovery and to the decrease in serum bilirubin concentration. It seems to be independent of the initial bilirubin concentration. In Dav the pattern returns to normal when the bilirubin concentration decreases from 26 to 11 mg. per 100 ml., while in Man this happens when the bilirubin falls from 3.6 to 0.4 mg. per 100 ml.

Usually the serum alkaline phosphatase concentration decreases with the bilirubin, but the correlation between the phosphatase and the lipid pattern is far from exact. No correlation can be established for the change in pattern and the changes in bromsulfalein excretion or cephalin flocculation or thymol turbidity tests. Our data are not sufficient to establish presence or absence of correlation between the lipid pattern and the urinary excretion of bile and urobilinogen. In Figure 1 are shown the patterns of change in a typical case of acute hepatitis.

Portal cirrhosis

In Table V are shown the data in patients with portal cirrhosis who have been listed in order of descending plasma cholesterol concentration. Listed separately are one case of hemochromatosis and five adolescent females, with the syndrome described by Bongiovanni and Eisenmenger (11) and characterized by high content of protein (up to 5.99 gm. per 100 ml. in Mar) in Fraction II.

The lipid deviations in this group are extremely variable. The total cholesterol concentration varies between the wide limits of 551 and 44 mg. per 100 ml. In twenty of the thirty-six patients the plasma cholesterol is less than the lower limit of normal and in four patients cholesterol values of less than 100 mg. per 100 ml. are observed. Generally, there is a decrease in cholesterol in Fraction IV + V + VI while that in Fraction I + III varies, depending upon the total plasma concentration. In the majority of cases the phospholipid varies with cholesterol as in normal subjects so that the ratios of cholesterol to phospholipid are not different from normal, but in nine of the patients low cholesterol-phospholipid ratios in the plasma and Fraction I + III are observed. In five of them free to total cholesterol ratios in the plasma are measured, and in four they are over 0.75. Two other patients have ratios over 0.70: in Alt the cholesterol-phospholipid ratios are normal, but in Ede they are low but not as low as in the patients discussed. In the other patients with portal cirrhosis the mean ratio of the free to total cholesterol is 0.50. In these patients with the abnormal lipid pattern serum bilirubin is usually elevated. Although Rob, Bur, and Car were in the terminal phase of their disease, the other patients were not regarded as having an immediately grave prognosis. In Guz and Rob the transient nature of these episodes is shown. With the disappearance of the intense jaundice the cholesterol-phospholipid and free to total cholesterol ratios return to levels seen in the remainder of the patients with cirrhosis.

In the cases of Table VI in which the Sperry-Schoenheimer determinations were carried out on the fractions of plasma, the observations are not sufficient in number to permit any general statement. It will be noted, however, that in biliary cirrhosis and in the acute stage of the acute hepatitis the cholesterol content of Fraction IV + V + VI is almost entirely unesterified, while in Fraction I + III some of the cholesterol is esterified.

TABLE V

Protein-lipid relationships during the course of portal (Laennec's) cirrhosis, hemochromatosis, and the cirrhosis of adolescent females

Name	Age	Sex	Duration of illness (*months*)	Date	Total serum bilirubin *mg. per 100 ml.*	Cholesterol				Phospholipid			Chol./P'lipid		
						Plasma *mg. per 100 ml.*	Fractions IV+V+VI *mg. per 100 ml.*	Fractions I+III *mg. per 100 ml.*	Free/Total cholesterol plasma	Plasma *mg. per 100 ml.*	Fractions IV+V+VI *mg. per 100 ml.*	Fractions I+III *mg. per 100 ml.*	Plasma	Fractions IV+V+VI	Fractions I+III
						Portal (Laennec's) cirrhosis									
Alt	39	M	5	8/14/52	14.8	551	52.4	504.0	0.73	474	83.5	375.0	1.16	0.63	1.34
Guz	51	M	1	9/16/53	23.6	181	27.4	149.1	0.81	334	46.6	282.0	0.54	0.59	0.53
				11/2/53	1.2	223	54.3	160.7	0.36	295	135.1	149.0	0.76	0.40	1.08
				11/25/53	1.3	306	44.1	250.5	....	374	93.0	116.9	0.82	0.51	1.33
Rob	36	F	5	2/20/53	30.2	120	12.5	103.2	0.75	213	25.8	182.8	0.56	0.48	0.57
				4/8/52	7.3	276	28.3	242.5	0.50	314	67.1	204.2	0.88	0.42	1.19
				5/6/52	3.5	314	54.3	....	0.42	337	....	....	0.93	....	....
				12/2/52	10.2	216	27.9	192.5	0.45	257	74.4	189.9	0.84	0.36	1.02
Gau	42	M	108	6/19/53	0.2	274	35.1	228.0	0.35	261	72.5	188.4	1.05	0.48	1.21
Sko	48	M	36	4/28/52	8.3	267	57.6	203.5	0.54	314	96.5	182.9	0.85	0.60	1.11
				6/9/52	20.4	193	30.9	153.7	....	216	38.2	139.2	0.90	0.81	1.10
Cam	62	M	4	1/29/52	2.4	244	57.1	178.9	....	...	....	....	....	....	....
Nei	51	F	48	10/11/51	3.7	246	29.2	207.5	....	270	70.2	187.0	0.91	0.42	1.11
Cur	45	F	1	2/13/53	2.6	236	57.1	173.8	....	301	129.5	160.0	0.79	0.44	1.09
Mar	25	F	19	11/29/51	4.6	227	15.7	199.0	0.44	268	48.9	209.0	0.85	0.32	0.95
Pri	57	M	48	6/1/53	1.4	220	57.0	150.7	0.50	268	133.2	126.0	0.82	0.43	1.20
Cra	65	M	30	8/21/51	2.7	211	59.6	150.1	....	279	127.5	133.0	0.76	0.47	1.13
Gue	42	M	24	8/13/52	2.2	210	39.7	163.0	0.44	250	85.2	136.2	0.84	0.47	1.19
Lie	55	F	3	8/9/51	0.8	202	25.3	174.9	....	256	69.8	128.5	0.79	0.36	1.36
Lew	36	F	1	2/3/53	5.0	192	32.4	152.0	0.61	268	77.8	180.0	0.74	0.32	0.85
Roh	60	M	12	10/14/52	3.2	170	29.9	135.2	....	304	183.0	212.0	0.56	0.36	0.64
Hog	69	M	18	6/1/53	1.7	163	50.6	106.2	0.49	208	118.2	90.9	0.78	0.43	1.17
Mah	54	M	24	4/22/53	13.8	163	20.8	136.0	0.60	292	59.5	220.0	0.56	0.35	0.62
Piz	53	M	15	6/1/53	...	161	38.8	114.0	0.52	220	130.7	110.2	0.73	0.37	1.04

TABLE V—*Continued*

Name	Age	Sex	Duration of illness (*months*)	Date	Total serum bilirubin *mg. per 100 ml.*	Cholesterol				Phospholipid			Chol./P'lipid		
						Plasma *mg. per 100 ml.*	Fractions IV+V+VI *mg. per 100 ml.*	Fractions I+III *mg. per 100 ml.*	Free/Total cholesterol plasma	Plasma *mg. per 100 ml.*	Fractions IV+V+VI *mg. per 100 ml.*	Fractions I+III *mg. per 100 ml.*	Plasma	Fractions IV+V+VI	Fractions I+III
Dvi	42	F	4	4/15/53	16.8	157	21.8	132.2	....	350	53.9	290.0	0.45	0.40	0.46
Fis	48	F	12	10/22/51	4.7	169	10.9	137.3	....	186	37.1	132.0	0.91	0.29	1.04
Cuy	69	M	1	9/11/51	2.3	138	22.5	113.8	....	...	....	....	....	....	....
Pod	33	F	24	6/2/52	2.6	136	10.2	120.1	0.59	172	29.9	124.9	0.79	0.34	0.96
Gib	47	M	6	5/28/51	6.1	141	20.2	113.5	....	...	....	....	....	....	....
Mak	23	F	23	9/12/51	3.4	142	33.3	96.0	....	...	....	....	....	....	....
Nel	51	M	6	8/26/52	1.0	122	32.0	89.4	....	168	67.0	74.5	0.73	0.48	1.20
Bus	44	F	48	9/19/51	7.8	120	18.7	94.6	....	200	52.1	145.7	0.60	0.36	0.65
				1/28/52	...	119	12.4	102.1	....	137	29.6	92.0	0.87	0.42	1.11
Rho	52	F	4	12/5/52	2.5	116	13.7	95.5	0.42	170	39.8	123.1	0.68	0.34	0.78
Fur	63	M	9	5/3/51	1.6	128	31.4	78.6	0.52	157	69.0	57.9	0.81	0.46	1.17
Bur	61	M	1	11/5/51	26.6	99	8.0	94.2	0.83	198	13.9	165.0	0.50	0.57	0.57
Vog	52	M	12	7/9/51	4.7	86	14.4	61.4	....	121	49.8	65.5	0.71	0.29	0.93
								Hemochromatosis						
Car	59	M	40	2/25/52	13.1	76	8.9	67.9	0.77	124	16.4	105.0	0.62	0.54	0.65
				3/11/53	13.6	44	2.3	41.2	....	85	4.2	76.3	0.52	0.54	0.54
								Cirrhosis of adolescent females							
Ede	28	F	96	9/25/51	11.9	162	20.6	139.5	....	...	....	....	....	....	....
				10/17/51	12.0	129	19.6	112.3	0.76	168	25.0	130.0	0.77	0.78	0.86
Bob	18	F	36	5/2/51	7.5	114	12.8	84.5	0.55	119	19.5	88.0	0.96	0.66	0.96
				5/21/51	9.2	148	18.2	116.0	....	...	....	....	....	....	....
Joh	16	F	9	7/10/51	21.4	119	24.6	83.5	....	185	70.4	88.0	0.64	0.35	0.95
Ste	16	F	18	7/10/51	4.6	82	4.6	63.5	....	152	8.7	137.0	0.54	0.53	0.46
Mar	23	F	6	11/18/52	7.7	79	9.9	66.4	0.48	122	29.8	87.1	0.65	0.33	0.76

TABLE VI

Free cholesterol, esterified cholesterol, and free to total cholesterol ratios in Fractions IV+V+VI and I+III

Name	Date	Cholesterol: Plasma Ester *mg. per 100 ml.*	Plasma Free *mg. per 100 ml.*	Plasma Free/Total	Fraction IV+V+VI Ester *mg. per 100 ml.*	Fraction IV+V+VI Free *mg. per 100 ml.*	Fraction IV+V+VI Free/Total	Fraction I+III Ester *mg. per 100 ml.*	Fraction I+III Free *mg. per 100 ml.*	Fraction I+III Free/Total
				Primary biliary cirrhosis						
Ros	12/21/51	17.0	1,023.0	0.90	15.0	816.0	0.98	115.0	167.0	0.89
Yaz	9/2/52	157.0	509.0	0.76	0	410.0	1.00	106.0	113.0	0.52
Whe	4/8/53	96.0	390.0	0.80	4.0	230.0	0.98	87.0	109.0	0.56
				Secondary biliary cirrhosis						
Sto	11/19/51	108.0	110.0	0.51	4.5	24.1	0.84	85.0	86.0	0.50
				Bile duct obstruction						
Zik	9/7/51	96.0	1,095.0	0.92	17.0	860.0	0.98	83.0	148.0	0.64
Smi	3/18/53	145.5	90.5	0.38	54.7	41.7	0.43	76.8	47.7	0.38
				Acute hepatitis						
Dav	3/31/52	37.0	161.0	0.81	0	51.3	1.00	131.0	120.0	0.92
	4/15/52	25.0	143.0	0.85	0	27.6	1.00	41.4	101.1	0.71
Stu	10/22/51	42.5	134.5	0.76	0	64.6	1.00	43.2	65.8	0.60
	11/1/51	129.5	61.5	0.32	20.8	7.0	0.25	100.6	45.4	0.31
	11/12/51	154.0	54.0	0.26	35.2	8.8	0.20	102.4	40.6	0.28
Lau	11/12/51	179.3	66.7	0.27	27.6	8.4	0.23	136.7	57.3	0.30
Saz	1/10/52	0	63.2	1.00	1.3	0.7	....	7.5	63.2	0.88
Ran	11/7/51	40.0	244.0	0.86	0	141.0	1.00	34.9	94.1	0.73
	11/15/51	138.0	193.0	0.58	1.2	94.0	0.89	91.4	81.6	0.47
Hoo	1/7/52	17.0	104.0	0.86	0	15.0	1.00	25.6	82.4	0.76
	1/23/52	60.0	124.0	0.67	3.6	27.0	0.88	42.0	99.5	0.70
	7/9/52	117.4	37.2	0.24	29.1	5.2	0.15	78.2	37.8	0.33
Ols	9/11/52	21.7	44.6	0.67	5.0	5.0	0.50	27.1	38.0	0.58
				Portal cirrhosis						
Rob	2/20/52	28.5	84.8	0.75	2.1	9.7	0.82	25.0	74.0	0.75
	5/6/52	145.1	106.6	0.42	21.3	15.5	0.42	104.0	62.9	0.38
Gau	6/19/52	165.4	87.6	0.35	20.2	6.2	0.23	127.7	65.8	0.34
Pri	6/1/53	88.3	89.2	0.50	24.4	26.0	0.52	63.4	61.5	0.49
Lew	2/3/53	77.1	120.5	0.61	4.6	26.9	0.85	78.3	75.2	0.48
Hog	6/2/53	55.3	53.3	0.49	24.3	15.3	0.39	39.2	33.2	0.46
Piz	6/1/53	58.8	64.0	0.52	21.9	17.0	0.44	40.6	47.2	0.54

Filter paper electrophoresis

Normal plasma has two principal lipid-staining components; namely, an intense deeply-stained spot occurs in the beta globulin area and another, less intense, in the area between alpha$_1$ globulin and albumin. In biliary cirrhosis, despite the high lipid content of the plasma, the alpha component is negligibly small and all of the stainable lipid appears to be present in the beta area. Similar patterns have been found in obstructive jaundice and acute hepatitis. With disappearance of icterus, stainable lipid then appears in the alpha area.

Fraction IV + V + VI from the plasma of a patient with biliary cirrhosis was subjected to paper electrophoresis, and all the lipids were found to migrate with the mobility of beta globulin, and when run on a starch block the fraction had a large single lipid peak with a cholesterol-phospholipid ratio of 0.50. The lipoprotein of Fraction I + III on filter paper also migrated to the beta area.

Ultracentrifugal flotation

In Table VII are shown comparisons of the separation made by ultracentrifugal flotation with those made by Cohn fractionation. In this table, the per cent of total cholesterol and phospholipid found in the bottom fraction (containing alpha lipoproteins) is compared with that found in Fraction IV + V + VI which ordinarily contains alpha lipoproteins. In one normal subject and in two patients with hypercholesterolemic xanthomatosis the correlation between the two techniques

is good. In Tad it was not quite so close. In three patients with biliary cirrhosis and in one with acute hepatitis there is much less lipid in the bottom fraction than in Fraction IV + V + VI. This correlates well with the comparable studies by filter paper electrophoresis.

DISCUSSION

In these patients with obstructive jaundice, the lipid composition of the lipoprotein-containing fractions differs markedly from that found in normal plasma and in the plasma from patients with atherosclerosis and related diseases. Furthermore, these lipoproteins have physical properties different from those ordinarily found. The lipoproteins in Fraction IV + V + VI as well as those in Fraction I + III have the electrophoretic mobility of beta globulins. The greater part of the lipoprotein in Fraction IV + V + VI has a density of less than 1.063 as does all the lipoprotein in Fraction I + III. The lipoproteins separated by the physical methods also differ in chemical composition from those usually found. Kunkel and Slater (9) have shown that the material migrating with the mobility of beta globulin on a starch block has a cholesterol-phospholipid ratio of 0.5 as contrasted to normal values of 1.26 for this peak. Similarly, the low density lipoproteins separated by ultracentrifugation have low cholesterol-phospholipid ratios.

At present, lipoproteins can be defined only by description of certain of their chemical and physical properties. On this basis it might be concluded that in obstructive jaundice there are abnormal lipoproteins in the plasma. It cannot be assumed, however, that these are newly synthesized compounds. Anfinsen (16) has pointed out that the abnormality might consist of an accumulation of lipoproteins which are normally present in only minute amounts. Another possibility is that the lipid-binding capacity of normal lipoproteins has been altered by unknown factors or as Byers, Friedman, Biggs, and Gunning (17) have suggested by accumulation of bile acids.

The evidence of the present study indicates that at least two types of these abnormal lipoproteins are present in patients with obstructive jaundice. One is soluble under the conditions of precipitation of Fraction I + III and therefore appears in Fraction IV + V + VI, while the other is insoluble and is separated with Fraction I + III. Both have cholesterol-phospholipid ratios considerably lower than those normally found in Fraction I + III. They differ in their ratios of free to total cholesterol: the cholesterol in Fraction IV + V + VI is almost entirely free (unesterified) whereas in Fraction I + III the free cholesterol constitutes from 50 to 80 per cent of the total. Snavely, Goldwater, Randolph, and Unglaub (10) by ultracentrifugation of plasma from patients with acute hepatitis also obtained a fraction in which all the cholesterol was unesterified.

These abnormal lipoproteins are present in large amounts in biliary cirrhosis, and are often found in both intra- and extrahepatic obstruction of the biliary tract. They accumulate constantly in the early stages of the jaundice of acute hepatitis when intrahepatic obstruction of finer biliary radicals may be a feature (18). They have also been found in cerain patients in whom jaundice is

TABLE VII

Comparison of ultracentrifuge fractions with Cohn fractions

Name	Diagnosis	Per cent of total cholesterol		Per cent of total phospholipid	
		Bottom fraction	Fraction IV+V+VI	Bottom fraction	Fraction IV+V+VI
Wil	Normal female	48.6	62.2	78.5	76.1
Mis	Hypercholesterolemic xanthomatosis	7.4	6.4	24.1	16.0
Hid	Hypercholesterolemic xanthomatosis	7.6	10.4	31.6	26.1
Tad	Hypercholesterolemic xanthomatosis	6.8	3.2	15.0	8.7
Pey	Biliary cirrhosis	3.4	85.2	5.0	81.5
Dor	Biliary cirrhosis	10.8	39.2	14.9	41.1
Bro	Biliary cirrhosis	7.0	38.6	14.4	51.3
Roj	Acute hepatitis	5.1	30.6	15.7	15.7

marked and biliary obstruction apparently present. In two of the cases in this series (Alt and Sko) intense jaundice developed without accumulation of abnormal lipoproteins. In one of them (Sko) the jaundice may have been hemolytic rather than obstructive. In the other, no explanation is offered for the lack of correlation. In patients whose portal cirrhosis is associated with only moderate degrees of jaundice, these lipoproteins are not found.

It appears that the concentration of these abnormal lipoproteins is related to the liver's synthetic capacity. Thus, in patients with early biliary cirrhosis in whom liver function is relatively unimpaired, high plasma concentrations of these lipoproteins are found. As the disease progresses and liver function is impaired, the concentrations fall. In hepatitis, where hepatic function is of course impaired, high levels of abnormal lipoproteins are not found. Data from electrophoretic and ultracentrifugal separations indicate that when the abnormal lipoproteins accumulate there is certainly depression of the alpha lipoprotein concentration and probably also of the normal beta lipoprotein concentration. In recovery from acute hepatitis the return of the normal lipoproteins is indicated by a return of the lipid in Fraction IV + V + VI to normal concentrations and by elevation of the cholesterol-phospholipid ratio in Fraction I + III.

Accumulation of lipoproteins of altered chemical composition offers an explanation of some of the deviations in plasma-lipid relationships of liver disease that are common to our own data and those of Man, Kartin, Durlacher, and Peters (3) and of Albrink, Man, and Peters (5). In all the observations there is a correlation between low plasma cholesterol-phospholipid ratios and high plasma ratios of free to total cholesterol. It is evident that if most of the plasma lipoproteins have low cholesterol-phospholipid ratios, the plasma itself will also have a low ratio. Since little of the cholesterol in these lipoproteins is esterified, the plasma free to total cholesterol ratio will be elevated. Albrink, Man, and Peters (5) noted that "the ratio of free to total cholesterol is rarely as abnormal in cirrhosis with little or no icterus as it is in obstructive jaundice, although the liver damage may be far greater." This is consistent with the concept that these high free to total ratios are a consequence of the production of abnormal lipoproteins rather than an indication of parenchymal liver damage as had been earlier suggested (19).

The alterations in plasma lipids encountered in obstructive jaundice in man can be reproduced to some extent in animals by ligation of the common bile duct. In rats, Chanutin and Ludewig (20) demonstrated increases in serum free cholesterol with no increase in ester cholesterol and an increase in phospholipid proportional to the increase in free cholesterol. Byers, Friedman, and Michaelis (21) found similar changes in cholesterol. They showed (22) that hepatectomy prevented the rise in cholesterol and that (23) the amount of cholesterol that accumulated in the plasma was greater than could be explained by failure of biliary excretion. Subsequently, Fredrickson, Loud, Hinkelman, Schneider, and Frantz (24) showed that this increase in serum cholesterol following bile ligation is accompanied by a significant increase in the rate of cholesterol synthesis by the liver. It may be that the abnormal lipoproteins present in this circumstance may alter the partition of cholesterol between liver and plasma, and that this partition may control the rate of cholesterol synthesis.

SUMMARY

In obstructive jaundice and in early acute hepatitis the lipid composition of the lipid-containing protein fractions is markedly deviant from that of normals and of all other diseases we have studied.

In Fraction IV + V + VI there is approximately twice as much phospholipid as cholesterol, just as is found normally in that fraction. However, almost all of the cholesterol in the fraction is unesterified. The lipoproteins in this fraction have the electrophoretic and ultracentrifugal properties of beta lipoproteins, whereas normally they are high-density alpha lipoproteins.

The lipoproteins in Fraction I + III, like those normally in this fraction, have the physical characteristics of beta lipoproteins. Their proportion of ester cholesterol is less than normal. The most striking difference from normal lies in their cholesterol-phospholipid ratios which may be as low as 0.5 by weight.

It is concluded that in biliary cirrhosis, in some cases of obstructive jaundice, and in early acute hepatitis there are at least two types of abnormal lipoproteins, the one found in Fraction IV + V + VI and the other in Fraction I + III.

During the recovery phase of acute hepatitis and following the removal of biliary tract obstruction, the abnormal lipoproteins are replaced by lipoproteins of normal composition.

The lack of correlation between the appearance of these lipoproteins and the degree of hepatic damage in cases of portal cirrhosis indicates that factors other than injury to the liver are implicated in their formation.

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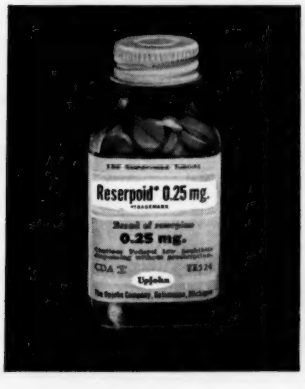

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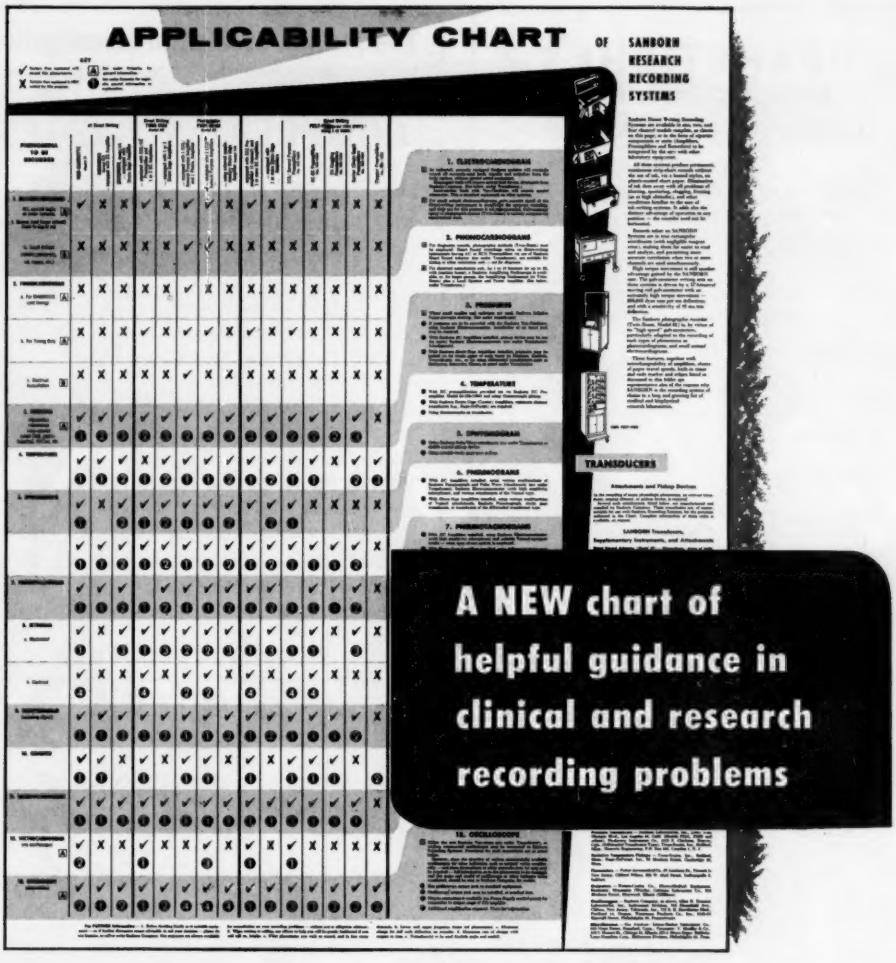
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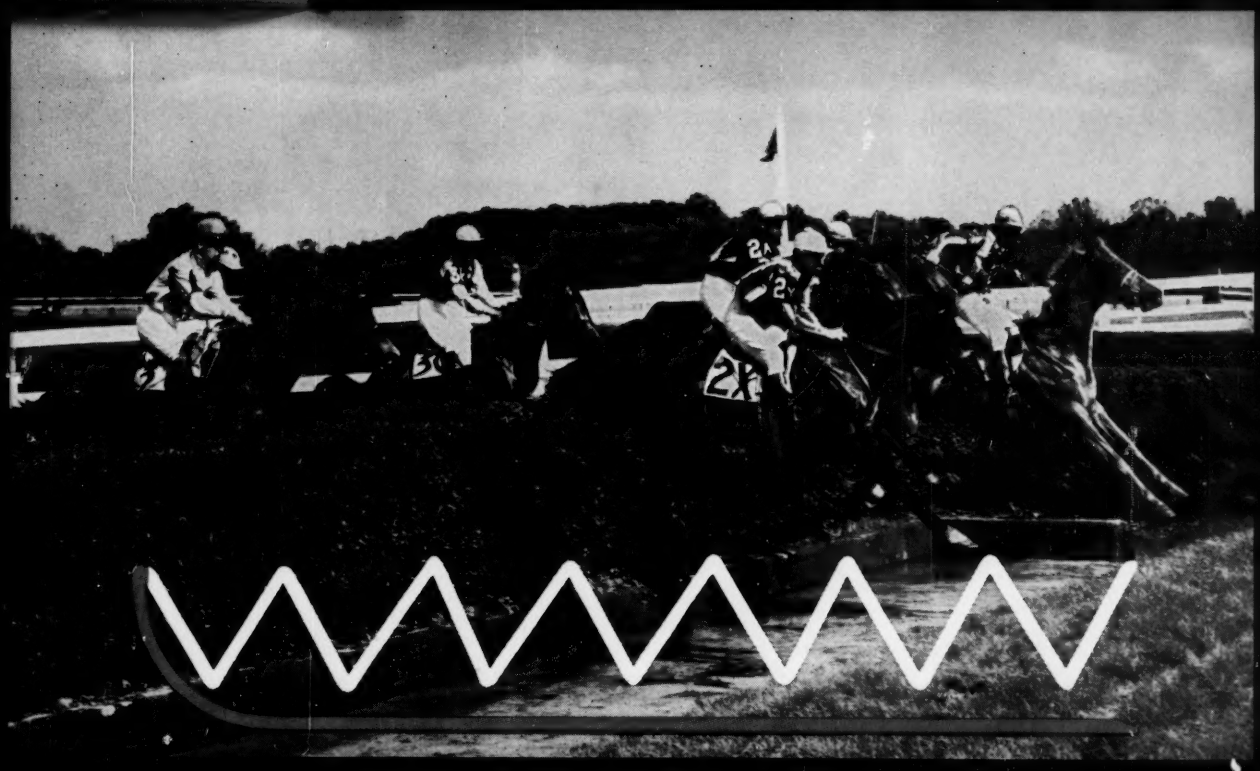

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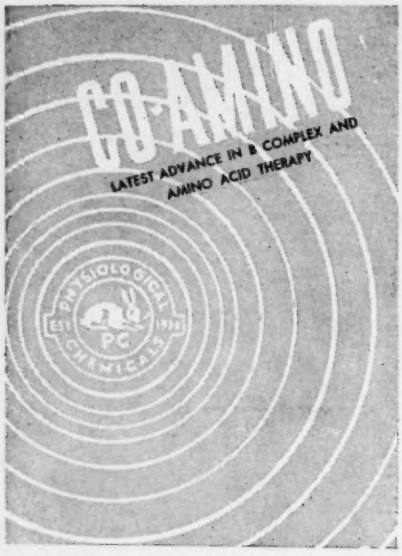

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The Treatment of Acute Infectious Hepatitis. Controlled Studies of the Effects of Diet, Rest, and Physical Reconditioning on the Acute Course of the Disease and on the Incidence of Relapses and Residual Abnormalities. THOMAS C. CHALMERS, RICHARD D. ECKHARDT, WILLIAM E. REYNOLDS, JOAQUIN G. CIGARROA, JR., NORMAN DEANE, ROBERT W. REIFENSTEIN, CLIFFORD W. SMITH, AND CHARLES S. DAVIDSON 1163

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THE TREATMENT OF ACUTE INFECTIOUS HEPATITIS. CONTROLLED STUDIES OF THE EFFECTS OF DIET, REST, AND PHYSICAL RECONDITIONING ON THE ACUTE COURSE OF THE DISEASE AND ON THE INCIDENCE OF RELAPSES AND RESIDUAL ABNORMALITIES [1]

By THOMAS C. CHALMERS,[2] RICHARD D. ECKHARDT,[3] WILLIAM E. REYNOLDS,[4] JOAQUIN G. CIGARROA, JR.,[5] NORMAN DEANE,[6] ROBERT W. REIFENSTEIN,[7] CLIFFORD W. SMITH,[8] AND CHARLES S. DAVIDSON WITH THE TECHNICAL ASSISTANCE OF MARY A. MALONEY, MERME BONNEL, MARIAN NIIYA, ALICE STANG, AND ANN McD. O'BRIEN

(From the Thorndike Memorial Laboratory, Boston City Hospital, The Departments of Medicine and Preventive Medicine, Harvard Medical School, and from The Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.)

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[1] This investigation was sponsored by the Commission on Liver Disease, Armed Forces Epidemiological Board, and was supported by a grant from the Office of The Surgeon General, United States Army, Washington, D. C.

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PART ONE

INTRODUCTION

1.1 *General remarks*

The usual patient with "catarrhal jaundice" was treated symptomatically and rather casually in the years prior to 1940. Emphasis was laid on strictly enforced rest in bed as a result of studies of the many military personnel who acquired acute infectious hepatitis during World War II. It was advocated by some that bed rest should be continued not only until all symptoms and signs had disappeared, but even until the major liver function tests returned to normal (1, 2). Later studies by others (3, 4) suggested that the period of bed rest need not be that prolonged. It remained to be clearly demonstrated that the enforcement of strict bed rest was necessary at any time during the acute phase of the illness.

The role of diet in hepatitis has aroused considerable speculation. Some investigators (5–7) advocated the forcing of a high-protein, high-calorie diet, with or without supplementary vitamins and amino acids. Others (8–10) were unable to demonstrate the effectiveness of one or more of these measures. Because of these divergent conclusions, a re-investigation of the effects of various dietary factors seemed indicated.

The incidence and importance of recurrent and chronic viral hepatitis have been the subject of many reports in the last few years (11–14). There have as yet been no well controlled studies in which attempts have been made to associate recurrent and residual abnormalities with the type of treatment administered during the acute illness.

These several pertinent problems relating to the proper treatment of patients with acute infectious hepatitis were still unsolved at the start of an epidemic of hepatitis among American military personnel in the Korean war.

The United States Army Hospital in Kyoto, Japan, was designated the "Hepatitis Center" for the Far East Theater of Operations in September of 1950. Since that time more than 4000 American military patients with hepatitis have been treated there. During the early phase of the Korean war over 1000 patients with well-established acute hepatitis were hospitalized at this Center at one time.

The 460 patients reported in these studies were all treated at the Hepatitis Center. They were then observed during their convalescence at the near-by Physical Reconditioning Center at Nara. Many of these patients were subsequently seen in a follow-up study either just before leaving the Far East Theater at Sasebo, Japan, or shortly after returning to the United States at their next duty station or their homes.

The "Hepatitis Study Team" which conducted these studies was organized in the spring of 1951. This team consisted of four civilian physicians (T. C. C., R. D. E., W. E. R., C. S. D.) and four

specially assigned Army doctors (J. G. C., N. D., R. W. R., C. W. S.). This group of investigators was accompanied to Japan by a chief technician (M. A. M.) and three research trained dietitians (M. B., M. N., A. S.). The final full-time member of the team coordinated the many administrative tasks (A. O'B.).

The main outline of the projected investigation was determined during the summer of 1951. The Hepatitis Study Team arrived in Japan in September of 1951 and remained there a full year. During the first three months the group critically analyzed the records of patients previously treated at the Hepatitis Center, set up a research laboratory, tested several diets, and trained 28 Japanese personnel to become dietetic and laboratory assistants. The investigations detailed in this report were completed over the next 10 months. The follow-up studies consumed approximately 9 months' additional time, or until July of 1953. In all, approximately two years were expended in these studies, exclusive of the time spent in the analysis and interpretation of the data.

It would not have been possible to accomplish these studies without the many contributions and full-scale cooperation of a large number of interested persons. Only those who by virtue of their command positions were responsible for the help contributed by their staffs, or who worked closely with the authors throughout the study, are mentioned below. In addition, specific contributions are acknowledged in footnotes to the text.

Grateful thanks are due Colin M. MacLeod, M.D., President, Armed Forces Epidemiological Board; Cecil J. Watson, M.D., Chairman, Commission on Liver Disease; William S. Stone, Colonel, M.C., Commandant, Army Medical Service Graduate School; Frank L. Bauer, Lieutenant Colonel, M.C., Director, Medical Division, Army Medical Service Graduate School; Victor Sborov, M. D., Director, Hepatic and Metabolic Division, Army Medical Service Graduate School; F. J. Knoblauch, Colonel, M.C., and A. J. Rapalski, Colonel, M. C., Administrators, Armed Forces Epidemiological Board; William E. Shambora, Major General, M. C., Chief Surgeon, Far East Command; James P. Cooney, Brigadier General, M.C., Surgeon, Japan Logistical Command; Charles L. Leedham, Colonel, M.C., and Francis W. Pruitt, Colonel, M.C., Consultants in Internal Medicine, Far East Command; Sterrett E. Dietrich, Colonel, M.C., Commanding Officer, and Robert S. Jordan, Lieutenant Colonel, M.C., Chief of Medicine, U. S. Army Hospital, Kyoto, Japan; Arthur E. White, Col. M.C., Commanding Officer, U. S. Army Hospital, Nara, Japan; and Herschel O. Griffin, Lieutenant Colonel, M.C., Commanding Officer, U. S. Army Hospital, Sasebo, Japan.

Finally, the authors would like to extend their thanks to those who contributed to the analysis of the data and preparation of the manuscript, namely: Dolores Dalo, Katherine Hendrie, Hazel Hitson, Tomoko Ikkai, Sheilla MacCrellish, Rita Nickerson, Joan Palmer, and Francis Y. Halsey.

1.2 *Preliminary epidemiological and clinical observations*

A report by Havens (15) of the hepatitis epidemic occurring among American troops in the Far East was available before the start of the study. The attack rate had risen steadily after the start of the Korean war, reaching a peak of 34 per thousand per year in Korea in February, 1951. Following this it fell slowly to a level around 8 per thousand per year, where it remained throughout the study. In contrast to the epidemic in postwar Germany (3), the onset of the illness was sudden in the large majority of patients and more closely resembled that of acute infectious hepatitis than homologous serum jaundice. Few wounded men who might have received plasma or blood were sent to the Hepatitis Center in Kyoto. Since most of the hepatitis patients had received immunizations or injections of antibiotics during the five months preceding admission, it was possible that some of them may have had homologous serum jaundice.

The treatment prescribed at the Hepatitis Center prior to the start of the studies followed closely the principles outlined by Capps and Barker (5). The patients were served a nutritious diet with Multivitamin and choline supplements, and were required to remain in bed until there were no residual symptoms or signs of hepatitis and until the bilirubin and bromsulphalein dye tests were normal. Then progressive ambulation was started cautiously, and if all went well, the patient was at full ambulation in about 2 weeks.

To gain an idea of the type of illness that had been treated prior to the start of the present stud-

ies, the data[9] on 2448 admissions to the Hepatitis Center during the previous year were reviewed. The average duration of hospitalization was 63 days. Four patients (0.2 per cent) died and 17 (0.7 per cent) were evacuated to the United States for "chronic hepatitis." Of 39 patients readmitted because of a suspected recurrence during this period, the diagnosis of hepatitis was established on the second admission in only seven. The disease was apparently continuous in five of the seven, so that only two patients had bonafide relapses or reinfections during the period of observation. Since all hepatitis patients in Southwestern Japan and Korea were by military directive to be transferred to Kyoto, it is unlikely that many patients with definite relapses had been treated elsewhere.

It was important to determine whether any measurable factors other than treatment influenced the course of the disease sufficiently to warrant a subdivision of the patients in the controlled studies before assignment to treatment groups. For this purpose a representative sample of 500 records were examined in greater detail. There was no evidence for an association between the duration of disease in the hospital and the patients' age (officers removed), race, place of onset, type of duty, history of previous hepatitis, coexistent intestinal parasites, duration of symptoms, dark urine or jaundice before admission; or whether the patient was withdrawn from duty before, at the time of, or after the discovery of jaundice. There was no relationship between previous alcohol consumption and duration of disease.

Among 80 patients with symptoms of less than 3 weeks' duration before admission, the duration of illness in the hospital varied directly with the level of the initial total serum bilirubin (correlation coefficient + 0.6). This relationship, in combination with estimates of the variability to be expected in duration of illness, was used to predict the number of patients required in the controlled studies in order to detect a treatment effect large enough to be of clinical interest.

1.3 *Outline of studies and summary of results*

Four studies were completed during the course of these investigations. The first three were controlled studies of the effects of diet, rest, and physical reconditioning on the course of acute infectious hepatitis. The fourth or follow-up study evaluated the incidence of relapses and residual abnormalities.

The first study was designed to determine: 1) Is strictly enforced bed rest required during the active stage of infectious hepatitis, or can patients be allowed up and about the ward as they feel fit? 2) Is the enforced feeding of a diet high in calories and protein and supplemented with choline and vitamins essential, or can patients eat only as much as they desire of a normally nutritious diet? Results of this study demonstrated: 1) Patients acutely ill with infectious hepatitis improved as rapidly when allowed to be up and about the ward at will as did patients kept at strictly enforced bed rest. 2) Patients forced to eat a diet supplying an abundance of calories, protein, and vitamins throughout hospitalization improved more rapidly than did those patients eating only what they chose of a normal diet.

The second study was planned to answer the following questions: How much and what kinds of food must patients with hepatitis, forced to eat throughout their hospitalization, consume in order to achieve maximum improvement during the acute phase of their illness? It was desired to ascertain whether an abundance of calories, protein, or supplements had any advantage over the required ingestion of an adequate or "normal" amount of each. The results demonstrated a small difference in favor of patients eating a high-protein diet.

The third study investigated this problem: How early can patients with infectious hepatitis, allowed reasonable activity on the ward during the active phase of their illness, safely undergo an active physical reconditioning program prior to discharge to duty? It was found that strenuous activity could be undertaken safely as soon as the total serum bilirubin was under 1.5 mg. per 100 ml. and the bromsulphalein retention was 5 per cent or less in 45 minutes.

In the fourth (follow-up) study patients were examined approximately one year after their initial treatment. No differences were discernible between patients allowed up and about at will and those kept at strictly enforced bed rest, or between patients who underwent early physical

[9] Kindly supplied by Capt. B. P. Summers, Registrar, and Lt. Col. R. S. Jordan, Chief of Medicine.

reconditioning and those whose return to full duty was gradual. Although there were no differences among the first study patients enforced to eat a nutritious diet and those who ate only as much as they desired, second study patients who received the high-protein and high-calorie diet showed a slightly higher incidence of mild residual abnormalities.

PART TWO

MATERIALS AND METHODS

2.1 *Selection of patients and allocation into treatment groups*

Patients from Korea and Southwestern Japan were transferred to the Hepatitis Center as soon as the diagnosis of hepatitis was made. The following criteria for acceptance were set up:

1) The patient had to be a male member of the Armed Forces of the United States below the grade of warrant officer.

2) The patient was required to have clinical jaundice or persistent dark urine, along with the probable diagnosis of infectious hepatitis as determined by one of the study's doctors from examination of the field medical record, brief history, and physical examination.

3) The duration of symptoms before admission had to be less than 22 days.

4) There could be no evidence on admission of the following diseases: acute malaria, dysentery, pneumonitis, infectious mononucleosis, or any condition requiring surgical treatment.

5) The accepted patients could not have received intravenous plasma or blood within six months of admission.

6) The patients could not have had infectious hepatitis within the last year.

All patients were seen immediately after arrival at the hospital by the study medical officer on duty and an admission card was filled out. After eligibility for the study had been determined by the above criteria and consecutive "study numbers" had been assigned to each patient accepted, envelopes containing a study number on the outside and a randomly assigned treatment group on the inside were opened and the patients were sent to the designated ward. Thus the physician *had no knowledge of the treatment the patient was destined to receive when eligibility for admission to the study was determined.*

2.2 *Details of patient care*

From the standpoint of statistical validity, it would have been desirable to have had each of the different treatments represented on the same ward. Only in this way could any unknown therapeutic effect of the patient's ward or doctor have been controlled. However, since it was reasoned that a patient's adherence to the prescribed regimen would depend for the most part on his confidence in his ward doctor's ability to provide the best available treatment for his speedy recovery and future health, the patients receiving different treatments had to be separated from each other as widely as possible. Therefore the four most widely separated of the eight wards in the hospital were chosen for the studies (Figure 1). Although the patients may have known that different methods of treatment were being used in the hospital, this was explained, when the question arose, with the statement that there were differing views as to the best treatment of hepatitis and that in such a situation it was important that the patient adhere to the regimen prescribed by his own doctor. For this reason it was impossible to rotate the doctors among wards or treatment groups in any one study.

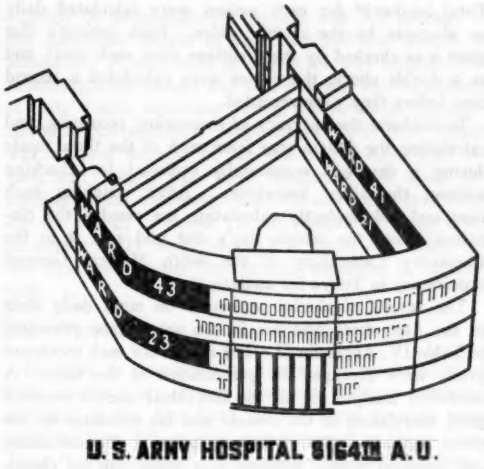

FIGURE 1

2.3 *Dietary procedures and validation of dietary calculations*

Working in collaboration with the Food Service Division of the Hospital, the research dietitians planned in detail the diets for each of the two treatment groups. Dietary sheets for each patient, showing the amount of each food item to be served, were mimeographed daily. On these the dietetic aides recorded the amounts originally served, refused, and the second servings. Standard servings were weighed on Hanson scales before each meal and meat portions and high-calorie foods such as desserts were weighed individually. Second helpings and uneaten portions were all weighed. If a patient vomited within 6 hours of a meal, half the value of the meal was subtracted from his intake. Sample intake sheets are reproduced in Tables II and III, Appendix II.

Total intakes[10] for each patient were calculated daily on abacuses by the dietetic aides. Each patient's diet sheet was checked by the dietitians after each meal, and as a double check, the values were calculated a second time before they were recorded.

To evaluate the accuracy of measuring, recording, and calculating the diets a tray from each of the three meals during a day was occasionally removed for checking without the aides' knowledge. After weighing each item and independently calculating the results, the dietitians mixed the sample day's diet and sent it to the Chemistry Laboratory of the 406th Medical General Laboratory in Tokyo for analysis.[11]

The results of checks performed on eight daily diets in the first study and six in the second are presented in Table IV, Appendix II. The results for each treatment group were averaged for presentation in the table. A statistical analysis of all the individual checks revealed good correlation of the protein and fat estimates by the three methods. The difference between the calculated and weighed protein contents was small, but on chemical analysis the protein averaged 13.6 grams more than the calculated content. The calculated fat content averaged 4 grams more than the weighed and 28.4 grams more than the chemically analyzed. If one assumes that the chemical analyses represent the "true" approximations, then roughly 9 per cent should be added to the protein content of each diet and 19 per cent subtracted from the fat content. It is possible, however, that as a result of incomplete ether extraction of the fat, the chemical analyses may have yielded low values.

2.4 *Methods for collection of data*

A. *Recording of admission history and physical examination.* When the study was set up it was hoped that by careful recording of historical data it might be possible to determine whether factors other than treatment had some effect on the striking variability in duration. Consequently many details of the patient's past history and present illness, which might conceivably have an influence on the duration of his hepatitis, were recorded in a standardized manner on an 8- by 11-inch punch card. (As in the preliminary observations no one of these variables was found to be significantly associated with the duration of illness as defined in this study.) For the back of the punch card, a semi-graphic recording of the present illness was devised. Employing a numerical grade of intensity, the daily occurrence of malaise, chills and fever, nausea and vomiting, diarrhea, upper abdominal pain, dark urine, light stools, and icterus was recorded. Also charted were the number of meals eaten each day after the onset of symptoms, the patient's activity on each day of his illness prior to admission to the study, the dates of his dispensary and hospital visits, the evacuation procedure, and the methods of treatment during hospitalization prior to transfer to the Hepatitis Center.

A complete physical examination was performed on admission and special attention was paid to the usual stigmata of hepatitis such as jaundice, hepatic size and tenderness, splenomegaly, and spider angiomata.

B. *Clinical observations in the hospital.* Each of the four ward doctors made daily rounds on his own ward and recorded clinical data daily during the patient's first week in the hospital, and twice a week thereafter. In order to insure standardization of clinical observations, group rounds were made once a week on successive wards. The following observations were recorded: degree of anorexia, nausea, upper abdominal pain, and malaise; number of episodes of vomiting and diarrhea each day; liver size and tenderness, palpability of spleen and presence of spiders; temperature, pulse, and weight; and the number of times the patient was out of bed.

C. *Laboratory procedures.* Liver function studies were performed at 3- or 4-day intervals throughout the period of observation. Besides the usual routine admission laboratory studies, each patient had two stool examinations for ova and parasites during the first week of hospitalization, and a heterophile agglutinin titer was performed three weeks after the onset of symptoms. All blood chemistry determinations were performed on blood samples taken between 7 and 8 a.m., with the patient fasting.

The work of the laboratory was supervised by one of the four ward physicians and the civilian chief technician. Ten Japanese chemists[12] were trained to perform the various tests. Although difficulties resulting from the different languages, training, and customs had to be overcome before the study could start, the laboratory work was performed efficiently throughout. At one time more than 2000 individual liver function tests were performed each week.

[10] Food values were taken for the most part from: Composition of Foods—Raw, Processed and Prepared, U. S. Dept. of Agriculture, Handbook No. 8, Washington, D. C., June, 1950.

[11] For the performance of the chemical analyses we wish to thank Col. R. L. Hullinghorst, Director of the 406th Medical General Laboratory, and his successor, Col. R. P. Mason, and Maj. Edward C. Knoblock, Chief of the Chemistry Laboratory. The following methods of analysis were used:

1. "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," 1945.
   a. Moisture—par. 27.7 and 28.2.
   b. Ash—par. 28.4 and 34.10.
   c. Fat—par. 28.6 and 27.25.
   d. Protein—par. 28.8 and 2.25.
2. "Analysis of Foods," A. L. Winton and K. B. Winton, 1945.
   a. Fiber—Henneberg Acid-Alkali Gravimetric Method—page 64.
   b. Carbohydrate by difference—page 63.

[12] The authors wish to thank especially Noriaki Wakizaka, M. D., and Koji Hamada, M.D., for their contributions to the laboratory work.

The routine liver function tests included the total and prompt direct (1-minute) serum bilirubin (17), cephalin flocculation (18), thymol turbidity and thymol flocculation (19), the zinc sulfate turbidity (20), and the serum cholinesterase activity (21). The bromsulphalein excretion test (22) was performed at weekly intervals after the total serum bilirubin had decreased to approximately 1.5 mg. per 100 ml. All liver function tests were performed in duplicate and final values represented averages of paired aliquots. When duplicates did not check within 5 per cent the test was repeated the following morning and all four values were averaged. All remaining serum was stored in a deep freeze until the termination of the study.

For the thymol turbidity test a reagent buffered at pH 7.8 as originally described by Maclagan (23) was employed. Although Mateer and co-workers (24) and Neefe, Gambescia, Gardner, and Knowlton (25) have indicated that thymol barbiturate reagent which is buffered at pH 7.55 probably provides a more sensitive procedure for detecting hepatic abnormality, the less sensitive technique was chosen to eliminate abnormal values of doubtful significance. All results of the thymol turbidity tests were reported in terms of Shank-Hoagland units (twice the Maclagan units).

For the bromsulphalein excretion test a dose of 5 mg. per kg. was employed. The body weight used in the calculation of bromsulphalein dosage was the average of the maximum weight at any time and the weight on admission. A maximum dose of 8 ml. was administered. Bromsulphalein dosage was kept constant despite changes in body weight occurring during the period of hospitalization or convalescence. This calculation of BSP dosage was adopted because of the variable weight loss before, and weight gain after admission, the latter depending on the treatment. Later it was found that varying the dosage by 0.5 to 1.0 ml. (equivalent to an error in weight of 11 to 22 pounds) in eight individuals did not significantly alter the per cent retention in 45 minutes.

2.5 *Normal values of liver function tests*

In order to determine the range of normal values of liver function tests among American Military personnel stationed in the Far East, fasting blood samples were collected from 279 enlisted men stationed at Camp Otsu, Japan,[13] and analyzed for prompt reacting (1-minute) serum bilirubin, total serum bilirubin, cephalin flocculation, thymol turbidity, thymol flocculation, zinc sulfate turbidity, and serum cholinesterase activity. All sub-

[13] The authors wish to thank Col. Dwight K. Foster, Commanding Officer, Camp Otsu, for his cooperation in making available the normal subjects.

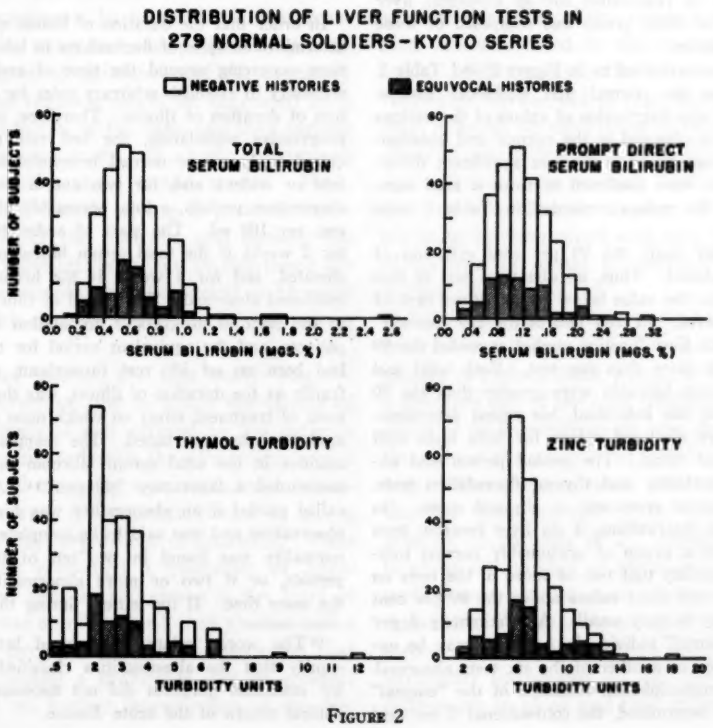

FIGURE 2

TABLE I

Liver function tests in 279 "normal" soldiers (Kyoto series)

	Mean	S.D.	S.E.	Normal limits 95%	Normal limits 99%
Total serum bilirubin—*mg./100 ml.*	0.64	0.26	0.016	1.04	1.62*
Prompt direct serum bilirubin—*mg./100 ml.*	0.12	0.05	0.003	0.20	0.26
Thymol turbidity—Shank-Hoagland units	3.5	1.6	0.095	5.5	6.5
Thymol flocculation				1+	2+
Cephalin flocculation—24 hours				1+	2+
Zinc turbidity—Kunkel units	7	2.4	0.14	11	13
Cholinesterase Δ pH per hour	0.90	0.16	0.01	0.60	0.44

* This value was not available for use in the definition of normal serum bilirubin for the first and second studies. Instead the value 1.50 mg. per 100 ml. described by Zieve and his associates (27) as the 99 per cent upper limit of normality was used.

jects were questioned for a past history of jaundice, hepatitis, infectious mononucleosis, exposure to hepatotoxins, blood or plasma transfusions, amebiasis, Weil's disease, malaria, and a family history of jaundice or anemia. Inquiry relating to present health was directed particularly to the discovery of symptoms associated with hepatitis, such as anorexia, vomiting, diarrhea, dark urine, and abdominal pain.

In analyses of the results of the tests, the individuals were separated into two groups. One group comprised all those without a past or present history of any factor which might be responsible for an abnormal liver function test. The other group was composed of those with positive histories.

These groups are referred to in Figure 2 and Table I, Appendix II, as the normal and equivocal groups. Since a review of the distribution of values of the various liver function tests obtained in the normal and questionably normal groups failed to disclose significant differences, the groups were combined to form a total sample from which the values presented in Table I were derived.

Throughout this study the 99 per cent criterion of normality is employed. Thus, an abnormal test is that found to fall above the value below which 99 per cent of the normals occurred. In only two of the 279 "normal" soldiers, values for liver function studies exceeded the 99 per cent level on more than one test. Both total and prompt direct serum bilirubin were greater than the 99 per cent values in one individual, but repeat determinations 2 days later disclosed values for both tests well within the normal range. The second person had abnormal thymol turbidity and thymol flocculation tests. He had no abnormal symptoms or physical signs. On the basis of these observations, if six liver function tests are performed on a group of presumably normal individuals, the probability that two or more of the tests on the same person will yield values above the 99 per cent level of normality is very small. Approximately 6 per cent of such "normal" individuals, however, may be expected to have one or another of the six tests abnormal.

Because the bromsulphalein retention of the "normal" soldiers was not determined, the conventional 5 per cent retention in 45 minutes was accepted as the upper limit of normal for this study. Since completion of the study, data have been made available to the authors indicating that the 99 per cent level lies between 10 and 14 per cent, depending on the weight of the individual (26). It is possible, therefore, that occasional patients diagnosed as having a prolonged acute course or a relapse on the basis of the bromsulphalein test, were in reality variants of the normal.

2.6 *Criteria for measurement of treatment effects*

In order that the duration of illness might be reliably determined in spite of fluctuations in laboratory observations occurring around the time of ambulation, it was necessary to establish arbitrary rules for the rigid definition of duration of illness. Therefore, in order to start progressive ambulation, the bed rest patient was required to have one normal bromsulphalein test (5 per cent or under) and, for two out of three semi-weekly observation periods, a total serum bilirubin less than 1.5 mg. per 100 ml. The start of ambulation was delayed for 2 weeks if the total serum bilirubin alone persisted elevated, and for 4 weeks if the bromsulphalein alone continued abnormal. The period of time from admission to the start of progressive ambulation for the bed rest patients, and the equivalent period for the patients who had been on *ad lib.* rest throughout, designated arbitrarily as the duration of illness, was the basic measurement of treatment effect on which most of the statistical analyses below are based. The reappearance of abnormalities in the total serum bilirubin or bromsulphalein constituted a laboratory "relapse."[14] A "relapse" was called partial if an abnormality was found on only one observation and was said to be complete if the same abnormality was found in two out of three consecutive periods, or if two or more abnormalities appeared at the same time. If the patient during the course of pro-

[14] The word "relapse" is placed between quotes to signify that the abnormalities classified as a "relapse" for statistical purposes did not necessarily constitute a clinical return of the acute disease.

gressive ambulation developed a partial "relapse," he was held at that level of activity until the next scheduled semi-weekly observation. If he developed a complete "relapse," he was transferred from his own ward, or from the Physical Reconditioning Center, to a fifth ward of the hospital on which all patients were treated alike and clinical and laboratory observations performed as during the acute study. The treatment of relapsed patients consisted of bed rest and the forcing of a moderately high-protein diet. When the original criteria for ambulation had again been attained, the "relapse" was designated as ended and the patient started back on progressive ambulation.

PART THREE

FIRST STUDY: THE RELATIVE EFFECTS OF DIET AND REST

3.1 *Design*

In this initial study the course of patients kept at strict bed rest throughout their acute disease was compared with the course of those allowed to be up and around on their hospital ward at will. Thus, the most conservative rest regimen was compared with the least conservative regimen which, since the patient could remain in bed if he wished, still might not be harmful. It was reasoned that if the strict bed rest patients improved faster, the degree of strictness and the necessary duration of the regimen might be suggested by a retrospective analysis of the data, and then further defined in a second study. If *ad lib.* rest proved adequate, two advantages would accrue. First, there would be a distinct saving in personnel necessary to care for the patients, and second, the duration of hospitalization would be shortened appreciably by elimination of the period of physical disability which usually follows prolonged rest in bed.

With respect to diet, the first study was designed as a screening one in which the effects of a nutritious hospital diet offered to the patients *ad lib.* were compared with the effectiveness of a diet in which all nutrients were included in abundant amounts and the patients forced to eat a minimum per day, regardless of anorexia. Thus the forced diet contained a minimum of 3,000 calories, 20 per cent or 150 grams in the form of protein, and was supplemented with choline and a Multivitamin preparation. It was reasoned that if merely providing a nutritious diet to patients was adequate, no further investigation of the dietary aspects would be necessary, and only if the forced diet proved superior would a second study be desirable to determine whether the course was shortened by the forced calories, the high protein content, the supplements, or all three.

FIRST STUDY
DESIGN OF TREATMENT GROUPS

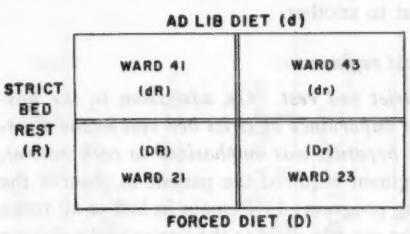

FIGURE 3

The two therapeutic trials summarized above were combined in a single study as outlined in Figure 3. Half the patients were on an *ad lib.* diet and half on a forced diet; similarly half, were on a program of *ad lib.* rest and half were kept in bed. This plan provided the means to determine whether the effect of one treatment factor, diet or rest, was influenced by the response to the other factor. For example, the effect of a diet might vary with the amount of rest, and if this were the case, it would be misleading to refer to an average effect of diet if the amount of rest were unspecified. If there were no such "interaction" between diet and rest, then the average effect of each factor could be determined with only half the number of patients as would be necessary if each were studied alone.

Two-hundred and sixty eligible patients were entered into the study in "blocks" of four. Each patient in a given block was assigned at random to a different one of the four treatment groups. The order of these assignments was determined before the start of the study from a table of random numbers. The patients were admitted in blocks of four for three reasons: 1) to maintain the same number of patients in all treatment groups; 2) to allow the number of patients to be increased if preliminary estimates of the variability of the duration of illness were too small; 3) to provide a means for controlling in the analyses of data the variability introduced by the unknown effects of differences in place and time of infection and fac-

tors in evacuation common to the patients in a given shipment, but expected to vary from one shipment to another.

3.2 *Rest regimens*

A. *Strict bed rest. On admission to the hospital the importance of strict bed rest in the treatment of hepatitis was emphasized to each patient.* This regimen required the patient to observe the following program: 1) Remain in bed at all times except for one trip daily to the latrine and a shower twice a week; 2) eat all meals in bed; 3) utilize all auxiliary services, including post exchange and library carts, at the bedside. Good doctor-patient relationships and constant vigilance on the part of the ward staff resulted in only rare infractions of the bed rest regimen.

Verification of adherence to the schedule was provided by inspection of the ward four to six times daily at irregular intervals. Results of these bed checks were recorded on a mimeographed sheet. If a patient was found out of bed at any other time by any of the ward personnel, this fact was also recorded on the bed check record. In addition, any patient found out of bed was reported to the ward doctor, who repeated the lecture on the importance of strict bed rest and requested complete compliance with the prescribed regimen.

Analysis of the data indicates that the criteria for strict bed rest were met by the great majority of patients (Figure 4). A small percentage failed to meet these criteria and were judged at a level of activity equivalent to or less than that entailed in free bathroom privileges or the eating of meals at the bedside.

B. *Ad lib. rest. No effort was made to control the level of activity of these patients except to prohibit boisterousness as a matter of ward discipline.* They were confined to their wards but permitted out of their rooms as much as they desired except for a one-hour rest period after each meal. Although allowed to be active, these patients were not required to perform tasks about the ward usually assigned to ambulatory military patients. Frequent inspections by the ward doctors were made to evaluate the patients' activity.

The levels of activity of the patients on the two regimens of rest were distinctly different (see Figure 4). From the first day of hospitalization, 45 per cent of the *ad lib.* group were out of bed more than the time involved in visits to the latrine and sitting up for meals. By the seventh day, 55 per cent of the patients, most of whom were still clinically jaundiced, were out of bed more than one-half of the day while only 7 per cent of the patients remained in bed except for latrine visits and meals. This marked difference in activity be-

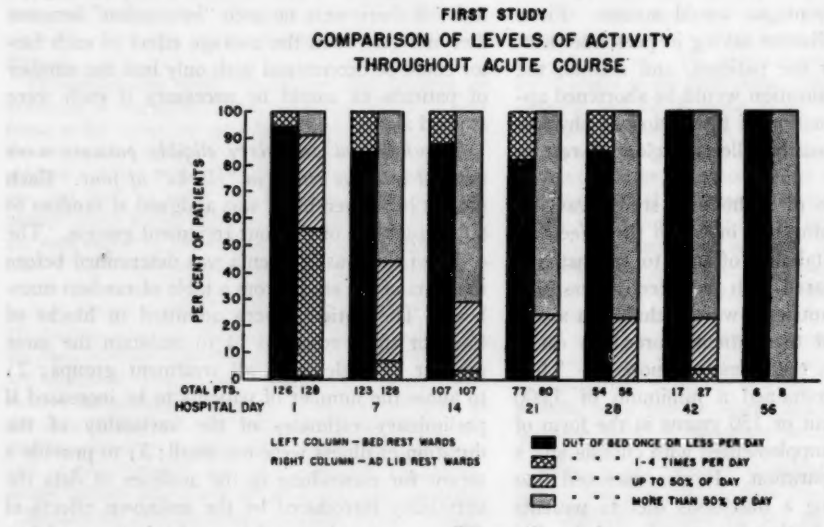

FIGURE 4

tween the strict and *ad lib.* rest wards was maintained throughout hospitalization and was immediately apparent to visitors to the wards.

3.3 *Dietary regimens*

A. *Forced high-protein diet. The potential importance of a forced intake of food in "curing" the disease was repeatedly emphasized to the patients.* They were required to eat a minimum of 3000 calories and 150 grams of protein each day, thus insuring a caloric protein proportion of at least 20 per cent. Fat and carbohydrate contents were allowed to seek their own level in each patient's diet depending upon his selection of food. The diet offered the patient on the menu was greater than this minimum value—at least 4000 calories and 200 grams of protein. Second helpings of all except high-calorie, low-protein foods were available. A high-protein milk drink was served between meals and at bed time. The patients also received choline dihydrogen citrate, 3.9 grams daily, and 2 Multivitamin tablets[15] three times a day. Candy and ice cream sundaes between meals were not allowed.

Patients who were unable to reach the established minimum were tube-fed. The doctors on the two forced-feeding wards met with the dietitians daily at 1 p.m. to decide if any patients required intubation, the amount of tube feeding necessary, and revision of the tube formula to conform with the food already eaten by the patient on that day. Glucose and saline solutions were occasionally given parenterally. Intravenous protein hydrolysate solutions were not used.

Six patients required tube feedings, two for 1 day only, two for 2 days, one for 3 days, and one for 4 days. It was found that the threat of intubation, or its use in one patient on a ward, was usu-

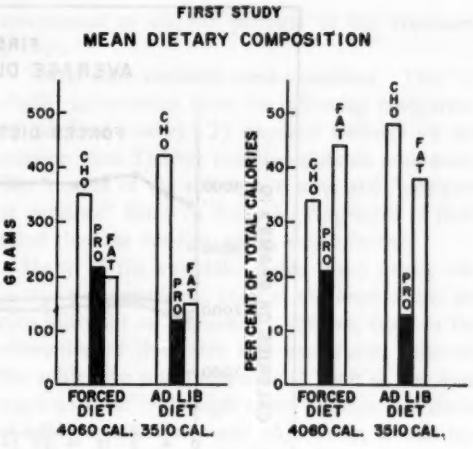

FIGURE 5

[15] Total daily dose supplied 15,000 U.S.P. units of vitamin A, 1200 U.S.P. units of vitamin D, 6 mg. of thiamin, 9 mg. of riboflavin, 225 mg. of vitamin C, and 60 mg. of nicotinamide.

TABLE II

Mean dietary intakes for treatment regimens—257 patients

	Diet				Rest			
	Forced		*Ad lib.*		Strict		*Ad lib.*	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Calories	4,060	402	3,510	621	3,730	535	3,840	638
S.E. of diff.		65				73		
P		<.001				.1		
Protein								
Grams	217	21	118	17	167	54	169	52
S.E. of diff.		2				7		
P		<.001				.7		
Per cent of calories	21		13		18		18	
Fat								
Grams	200		150					
Per cent of calories	44		38					
Carbohydrate								
Grams	348		422					
Per cent of calories	35		49					

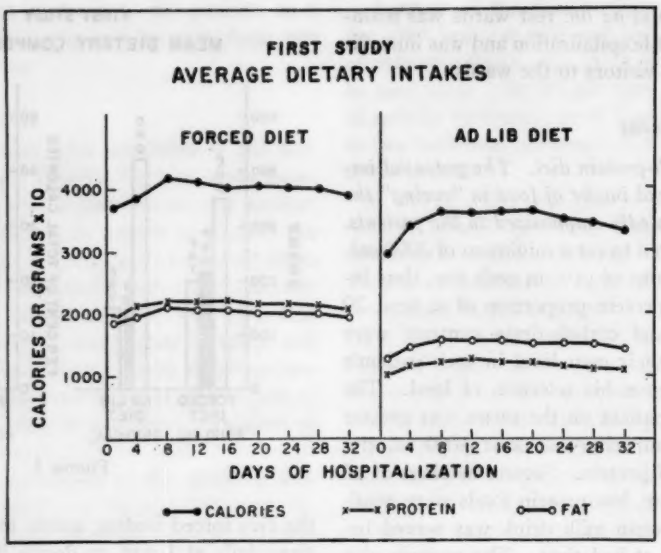

FIGURE 6

ally sufficient to persuade the rest of the anorectic patients to eat the required amount.

B. *Ad lib. diet. The possible benefits of food in the treatment of hepatitis were never mentioned to these patients.* They were served the standard Army hospital diet and no attempt was made to maintain any caloric or food consumption level. Patients were offered between-meal nourishments of fruit juices only. They could order sundaes, ice cream, and candy bars *ad lib.*, through the dietetic aides. Patients admitted with nausea and vomiting received fluids parenterally as necessary for purposes of maintaining hydration and electrolyte balance.

C. *Analysis of dietary intakes.* In Figure 5 are presented the mean intakes for each of the dietary groups in grams and per cent of total calories, from the time of admission to the start of convalescence. The protein differences were striking and the caloric differences small but significant (Table II). The fat content of the forced diet was appreciably higher than that of the *ad lib.* diet, the carbohydrate content lower.

The average intakes at the two dietary levels from admission to the start of convalescence are presented in Figure 6. Both rose somewhat in the first week, but the rise was greater in the *ad lib.* group. Both fell slowly after the first 8 to 12 days.

Since the intakes were *ad lib.* except for the required minimum in the forced group, the individual ranges were great. Figures 7 and 8 present

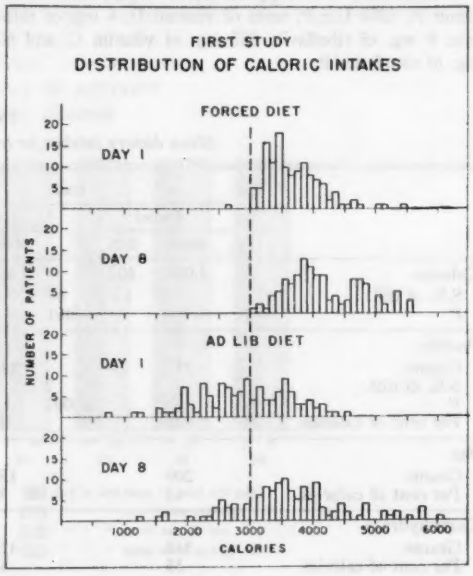

FIGURE 7

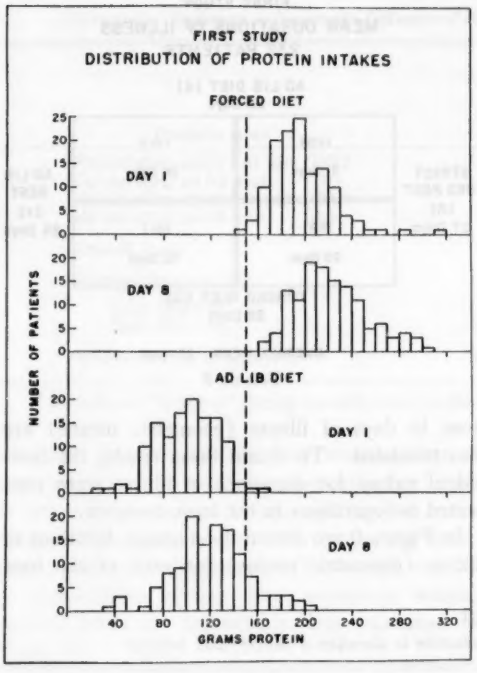

FIGURE 8

the distributions of intakes of calories and protein on the first and eighth hospital days. The range on the eighth day was representative of the intakes from that time on. On the forced-fed wards on day 1 only two patients fell below the prescribed minimum of 3000 calories and 150 grams protein. On the *ad lib.* wards, on the other hand, the range on day 1 was from 700 to 4500 calories, and from 20 to 160 grams of protein; on day 8 from 1200 to 6000 calories and from 30 to 200 grams of protein.

3.4 *Effectiveness of the randomization*

It was necessary to establish whether or not the four treatment groups were comparable with respect to the many variables which might influence the duration of illness, other than the treatments *per se*. A few of these variables were controlled by the criteria used in the selection of patients for the study. The distributions of all of the others, both known and unknown, were left to the play of chance by the use of random numbers for the assignment of eligible patients to the treatment groups.

Thirty-four variables were examined. They included information from the following categories: 1) Medical history; 2) physical findings on admission; and 3) liver function tests on admission. The results of the chi square tests and "analyses of variance" listed in Table V, Appendix II indicated that *the randomization was effective.*

Many of the variables tested were purely objective measurements, such as the level of the serum bilirubin on admission. Others, such as the estimation of liver size and tenderness, reflected the subjective interpretations of each of the four ward doctors. Although every attempt was made to achieve uniformity and objectivity, it was apparent early in the course of the analyses that certain significant differences could be attributed to systematic variations in the interpretation of historical facts and clinical signs and symptoms by the individual ward doctors.

The 34 variables were classified, without prior knowledge of the results of significance tests, into two groups—those in which a "doctor effect" seemed likely to appear, and those in which such effects seemed most improbable. For those 12 variables in which a "doctor effect" was considered improbable, there were no significant differences between the treatment groups. However, there were significant differences for 10 of the 22 variables that were amenable to subjective interpretation. Although these results did not affect the validity of the randomization, they did emphasize the inadequacies of clinical studies in which reliance for the measurement of treatment effects is placed primarily on symptoms and signs.

3.5 *Elimination of patients from the study*

The data on 7 of the 260 patients admitted to the study were removed from the principal analyses (Table VII, Appendix II). Three patients admitted to the study were subsequently found not to have infectious hepatitis. Their final diagnoses were, respectively, infectious mononucleosis, lymphogranuloma venereum, and psychoneurosis. Data on these three patients were removed from all but one of the analyses.

In the case of four other patients, the duration of illness was in doubt. Two of these patients left

the hospital before convalescence, one for an emergency leave and one for special treatment of an acute psychosis. The laboratory findings on the other two patients did not permit a distinction between a short initial illness with a long relapse and a long initial illness without relapse. The data for these four patients were excluded from all but one of those analyses which utilized the durations of illness.

In order to simplify certain analyses it was necessary to have the same number of patients in each of the four treatment groups. To accomplish this, each block of four patients which included any of the above seven patients was removed from the data, leaving 232 patients for these analyses.

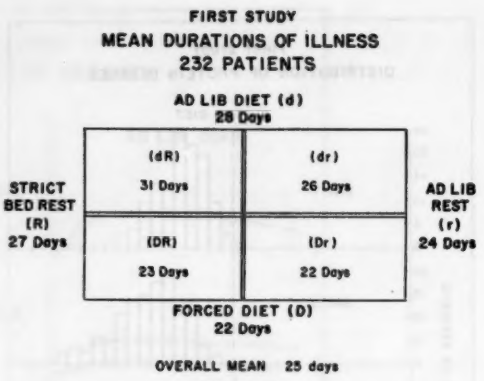

FIGURE 9

3.6 *The effects of treatment on duration of illness*

For both clinical and statistical reasons (see Appendix I, Sec. 3), basic results of the first study are presented as percentage reductions in duration of illness, although the absolute reductions in days of illness (geometric means) are also tabulated. To obtain these results the individual values for durations of illness were converted to logarithms in the basic analyses.

In Figure 9 are shown the average durations of illness (geometric means) for each of the four

TABLE III

First study: Responses to treatment: Percentage reduction in duration of illness—232 patients

Factor		Average response	Response with: Diet: Forced	Diet: *Ad lib.*	Rest: Strict	Rest: *Ad lib.*
Diet	Treatment comparison	(D) − (d)			(DR) − (dR)	(Dr) − (dr)
	% reduction (Absolute difference)*	−22% (−6 days)			−29% (−8 days)	−17% (−4 days)
	95% confidence limits	−14% to −30%				
Rest	Treatment comparison	(R) − (r)	(DR) − (Dr)	(dR) − (dr)		
	% reduction (Absolute difference)*	−10% (+3 days)	−4% (+1 day)	−16% (+5 days)		
	95% confidence limits	−0.1% to −19%				

Treatment levels: D—forced diet; d—*ad lib.* diet; R—strict bed rest; r—*ad lib.* rest.

* The sign of each absolute difference indicates the favorable treatment.

TABLE IV

First study: Responses to treatment: Logarithms of ratios of geometric mean durations—232 patients

Factor		Average response	Response with: Diet: Forced	Diet: *Ad lib.*	Rest: Strict	Rest: *Ad lib.*
Diet	Treatment comparison	(D)/(d)			(DR)/(dR)	(Dr)/(dr)
	Logarithm of ratio	−0.1085			−0.1370	−0.0801
Rest	Treatment comparison	(R)/(r)	(DR)/(Dr)	(dR)/(dr)		
	Logarithm of ratio	+0.0461	+0.0746	+0.0177		
Standard error of response		±0.0232		±0.0328		

TABLE V

First study: Responses to treatment: Influence of various definitions of the duration of illness—232 patients

Treatment group	Mean duration of illness from admission to: Convalescence (days)	Convalescence (weeks)	First normal TSB (days)	Last abnormal* TSB or BSP (days)
Forced diet, strict bed rest (DR)	26	3.1	22	30
Forced diet, *ad lib.* rest (Dr)	25	3.0	21	28
Ad lib. diet, strict bed rest (dR)	34	4.3	25	41
Ad lib. diet, *ad lib.* rest (dr)	30	3.7	25	35
Overall mean	29	3.5	23	34
Average response to:				
Diet (D) − (d)	−7	−1.0	−3	−9
Rest (R) − (r)	+2	+0.3	0	+4
Standard error of response	±1.6	±0.2	±1.2	±2.7

* Includes all "relapses" during convalescence in the hospital and at the Physical Reconditioning Center.

treatment groups; for the four combinations of two groups each of which represents the two levels of rest and two levels of diet; and for the entire study. The effects of diet and rest on the duration of illness are presented in Tables III and IV as comparisons among these geometric means, derived from the logarithmic data presented in Table IV.

The average response to diet was a 22 per cent or 6 days' reduction in duration of illness achieved by the forced diet relative to the ad lib. diet. This reduction is statistically significant at the 0.01 per cent level. The 95 per cent confidence limits indicate that the "true" effect of diet might be anything from a 14 per cent to a 30 per cent reduction in duration of illness in favor of the forced diet. This dietary effect was similar for patients both on strict bed rest and on the *ad lib.* rest regimen. Thus there was no significant interaction between rest and diet.

The average response to rest was a 10 per cent or 3-day reduction in duration of illness in favor of the ad lib. rest program. This result is statistically significant at the 5 per cent level, but the confidence limits show that the "true" reduction is probably not greater than 19 per cent and may well be as small as 0.1 per cent.

Since the definition of duration of illness chosen in the design of this study was necessarily arbitrary, it was important to examine the data retrospectively to determine the influence of different definitions upon the results. These results are

TABLE VI

First study: Treatment groups compared according to various methods for handling "eliminated patients": mean duration of illness from admission to convalescence in days

Treatment group	Blocks containing "eliminated patients" removed	"Eliminated patients" removed	All patients included
Number of patients	232	253	260
Forced diet, strict bed rest (DR)	26	26	26
Forced diet, *ad lib.* rest (Dr)	25	25	25
Ad lib. diet, strict bed rest (dR)	34	35	35
Ad lib. diet, *ad lib.* rest (dr)	30	30	30
Average response to:			
Diet (D) − (d)	−7	−7	−7
Rest (R) − (r)	+2	+3	+3
Standard error of response	±1.6	±1.6	±1.6

TABLE VII

First study: Occurrence of complete bromsulphalein "relapses"—253 patients

Place and time of occurrence	Number of "relapses"	Mean duration of "relapses" *days*
Hospital, first week of convalescence	6	28
Hospital, 2nd week or later in convalescence	9	40
Physical Reconditioning Center	2	21
Total "relapses"	17	33

tabulated in Table V[16] for the following definitions of "duration of illness"; 1) days from admission to convalescence; 2) weeks[17] from admission to convalescence; 3) days from admission to the first two "normal" total serum bilirubins (1.5 mg. per 100 ml.); and 4) days from admission to the last abnormal total serum bilirubin or bromsulphalein retention. Comparisons of the average responses to diet and rest with their standard error indicate that the statistically significant reduction in duration of illness produced by the forced diet is not appreciably influenced by these changes in definition of duration of illness. However, in none of these subsidiary analyses does the small response in favor of *ad lib.* rest reach the 5 per cent level of significance.

To determine whether the removal of patients from the basic data had influenced the results, the effects of diet and rest were obtained from three different analyses as follows (Table VI[16]): 1) with the 7 blocks (4 patients each or 28 patients) containing the "eliminated patients" removed; 2) with only the 7 "eliminated patients" removed; and 3) with the 7 patients left in and their durations of illness estimated when necessary. It is apparent that the results were not influenced by the manner in which the problem of "eliminated patients" was handled.

3.7 *Laboratory relapses before return to duty*

In 17 of the 253 patients (7 per cent), equally distributed among the treatment groups, the bromsulphalein test returned to a persistently abnormal level (for an average of 33 days) after the start of convalescence. In 30 other patients only one bromsulphalein test was abnormal during convalescence and in eight patients the total serum bilirubin was abnormal for one determination (partial "relapse").

There were thus 17 complete laboratory "relapses," all established by bromsulphalein elevations and occurring predominantly in the first few weeks of convalescence (Table VII). Two of the 17 patients complained of transient mild malaise. Only 1 of the 17 had concomitant reappearance of hepatomegaly and in 1 the liver became transiently tender. The total serum bilirubin rose transiently in 2 of these 17 patients (by 0.51 and 1.02 mg. per 100 ml., respectively), but only one determination in each of the 2 patients was above normal. In only 5 of the 17 was the bromsulphalein retention above 10 per cent during the "relapse." In these 5 and in 2 of the remaining 12 there were distinct rises in the zinc and thymol turbidity tests.

The distribution of "relapses" among treatment

[16] For purposes of technical simplicity the data summarized in Tables V and VI were not converted to logarithms before analysis, and the results are expressed as arithmetic means. They are slightly larger than the corresponding geometric means.

[17] Since the bromsulphalein test was performed once a week only, it was thought wise to check the results after the individual durations of illness had been converted from days to weeks.

TABLE VIII

First study: Effects of treatment on occurrence of "relapses"

Patients	Diet				Rest			
	Forced		*Ad lib.*		Strict		*Ad lib.*	
	No.	%	No.	%	No.	%	No.	%
Number of patients	128		125		126		127	
Without "relapse"	106	83	92	74	97	77	101	80
With partial "relapse"	15	12	23	18	19	15	19	15
With complete "relapse"	7	6	10	8	10	8	7	6
	P = .20				P = .75			

groups is presented in Table VIII. There is no evidence of any treatment effect.

3.8 *Other effects of treatment on laboratory tests*

There was a significantly more rapid fall of the mean serum bilirubin in the forced-diet groups than in the ad lib. diet groups. There was a tendency for the flocculation and turbidity tests to be more abnormal at the end of the acute illness in the forced-diet group. No effects of rest on these measurements were apparent.

A. *Mean serum bilirubin trends.* Since laboratory tests were performed at twice-weekly intervals it was possible to construct curves of the means over the first two hospital weeks. A longer period could not be examined in this manner because an increasing number of patients recovered from their illness after 2 weeks. The time intervals chosen for the curves were the following: 1 to 2, 3 to 6, 7 to 10, and 11 to 14 days after admission. When there were two determinations in any one period they were averaged. In each treatment group from one to three patients without at least one determination in each period were omitted from the analyses.

The curves presented in Figure 10 show a more rapid fall in both total and prompt direct serum bilirubins among the forced-diet patients and no difference between the rest groups. The sharper fall in the forced-diet patients is apparent within the first 3 to 6 days after admission. The falls between the admission and 7 to 10-day values are presented in Table IX, and the difference in fall of the total serum bilirubin is significant in the diet comparison.

In addition, it was found that significantly more patients on the *ad lib.* diet had rising total and prompt direct serum bilirubins after admission to the hospital (14 per cent *vs.* 4 per cent and 12 per cent *vs.* 4 per cent, respectively). Detailed data

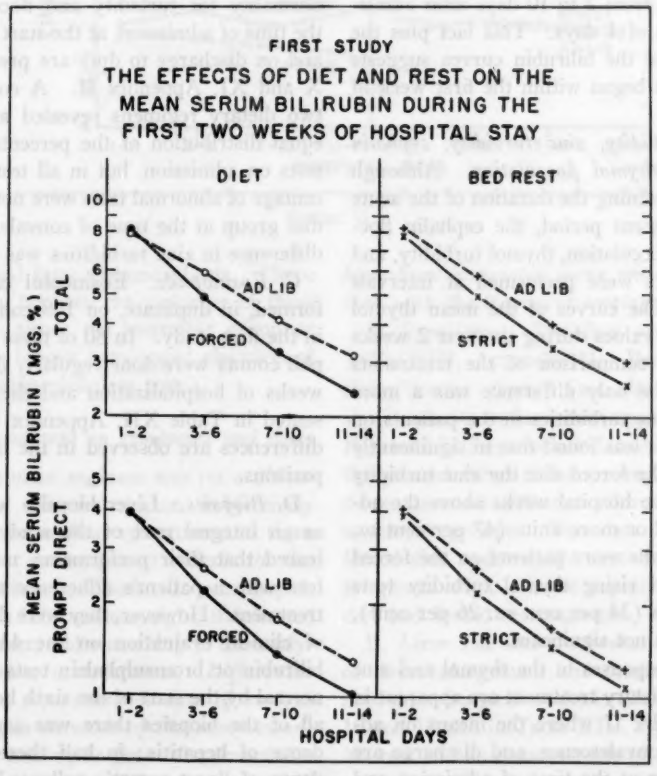

FIGURE 10

TABLE IX

First study: Mean fall in serum bilirubin during the first 7 to 10 days of each treatment regimen

	Diet			Rest		
	Forced	*Ad lib.*	P	Strict	*Ad lib.*	P
Number of patients	127	123		126	124	
Total serum bilirubin (Mg. per 100 ml.)						
Admission value	8.20	7.98		7.88	8.30	
Mean fall	4.91	3.82	.04	4.42	4.33	0.9
S.D.	3.93	4.37		4.06	4.32	
Prompt direct bilirubin (Mg. per 100 ml.)						
Admission value	4.02	3.91		3.92	4.01	
Mean fall	2.59	2.14	0.1	2.40	2.35	0.9
S.D.	2.09	2.31		2.17	2.26	

are presented in Table VIII, Appendix II. It should be noted that in the 23 patients with rises of 1 mg. per 100 ml. or more in total serum bilirubin and in the 21 with rises of 0.5 mg. per 100 ml. or more in the prompt direct serum bilirubins, the rises appeared from 2 to 10 days after admission, with a mean of 4 days. This fact plus the early divergence of the bilirubin curves suggests that the diet effect began within the first week of treatment.

B. *Thymol turbidity, zinc turbidity, cephalin flocculation, and thymol flocculation.* Although not employed in defining the duration of the acute illness or convalescent period, the cephalin flocculation, thymol flocculation, thymol turbidity, and zinc turbidity tests were performed at intervals on all patients. The curves of the mean thymol and zinc turbidity values during the first 2 weeks are presented for comparison of the treatments in Figure 11. The only difference was a more rapid fall of the zinc turbidities in the patients on the *ad lib.* diets. It was found that in significantly more patients on the forced diet the zinc turbidity rose in the first two hospital weeks above the admission value by 2 or more units (47 per cent *vs.* 30 per cent). While more patients on the forced diet also exhibited rising thymol turbidity tests by 1 or more units (34 per cent *vs.* 26 per cent), this difference was not significant.

The different responses in the thymol and zinc turbidity tests to dietary treatment are apparent in Table IX, Appendix II where the means on admission, start of convalescence, and discharge are presented. Similar at the time of admission and discharge, the means of both tests were significantly higher at the start of convalescence in the forced-diet group.

The percentages of patients in the first study with values above the 99 per cent upper limit of normality for turbidity and flocculation tests at the time of admission, at the start of convalescence and on discharge to duty are presented in Tables X and XI, Appendix II. A comparison of the two dietary regimens revealed an approximately equal distribution of the percentage of abnormal tests on admission, but in all tests a greater percentage of abnormal tests were noted in the forced-diet group at the time of convalescence (only the difference in zinc turbidities was significant).

C. *Eosinophils.* Eosinophil counts were performed, in duplicate, on 108 consecutive patients in the first study. In 80 of these patients, eosinophil counts were done regularly during the first 2 weeks of hospitalization and these data are presented in Table XII, Appendix II. No striking differences are observed in the diet or rest comparisons.

D. *Biopsies.* Liver biopsies were not planned as an integral part of the study because it was feared that their performance might often interfere with a patient's adherence to his prescribed treatment. However, they were done for purposes of clinical evaluation on the 43 patients whose bilirubin or bromsulphalein tests had not reached normal by the start of the sixth hospital week. In all of the biopsies there was some residual evidence of hepatitis; in half there was some evidence of "post-necrotic collapse," and in 37 per

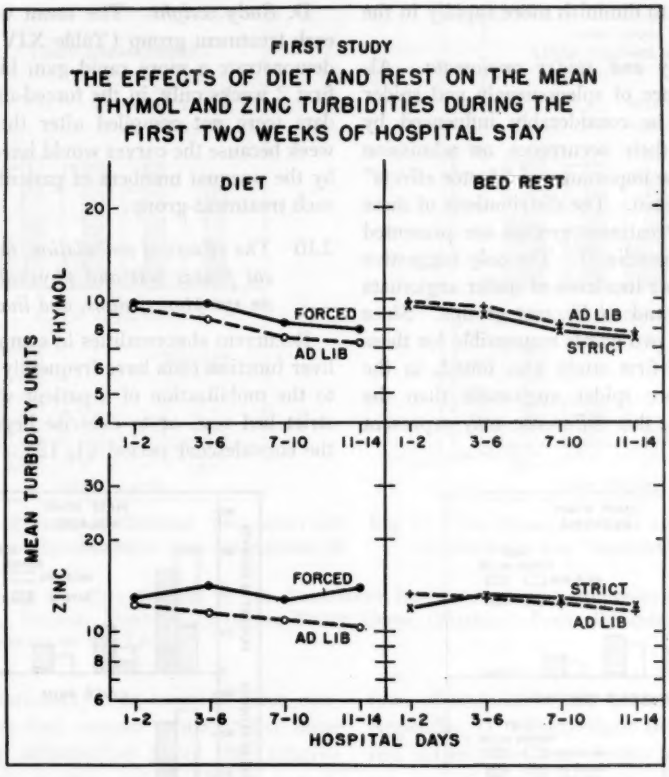

FIGURE 11

cent there was mild fatty metamorphosis. There was no association between the incidence of these abnormalities and either the treatment group or the reason for the biopsy (prolonged elevation of the bilirubin or bromsulphalein tests).

3.9 *Effects of treatment on symptoms and signs*

Since each treatment regimen was on a different ward with a different doctor, subjectively collected information such as symptoms and signs were given little or no weight as criteria for the evaluation of treatment effects. It was hoped, however, that such information might, if collected in a standard manner, add something to the analyses of the responses to treatment. *Differences were noted in the disappearance rate of various symptoms and signs in most of the comparisons made. Although tending to favor the forced-diet and ad lib. rest groups the differences may well have been associated more with "doctor effects" than with the treatment employed.*

A. *Symptoms.* The data are summarized in Table XIII, Appendix II. Because of the possible "doctor effects" (see also Section 3.4) no significance tests have been applied to the results. Figures 12–15 are included in the text to illustrate the general trend of symptoms and the fact that there were no striking differences between the treatment groups. The data suggest that the forced-diet and *ad lib.* rest patients lost their symptoms slightly more rapidly than the *ad lib.* diet and forced-rest patients.

B. *Liver size and tenderness.* The occurrence of liver enlargement and tenderness during the first three hospital weeks is illustrated in Figures 16 and 17 and in Table XIV, Appendix II. *Ad lib.* rest had no demonstrable effects upon the size and tenderness of the patients' livers. Liver en-

largement seemed to diminish more rapidly in the forced-diet group.

C. *Splenomegaly and spider angiomata.* Although the incidence of splenomegaly and spider angiomata might be considerably influenced by "doctor effects," their occurrence on admission was so rare that the importance of "doctor effects" could not be evaluated. The distributions of these abnormalities by treatment groups are presented in Table XIV, Appendix II. The only suggestive difference is a higher incidence of spider angiomata in the forced-diet and *ad lib.* rest groups. Since the doctors on the two wards responsible for these differences in the first study also found, in the second study, more spider angiomata than the other two doctors, this difference may represent a "doctor effect."

D. *Body weight.* The mean weight gains for each treatment group (Table XIV, Appendix II) demonstrate a more rapid gain in weight, in the first 2 weeks only, in the forced-diet group. The data were not compiled after the third hospital week because the curves would have been distorted by the unequal numbers of patients recovering in each treatment group.

3.10 *The effects of ambulation, the "pack" physical fitness test and physical reconditioning on symptoms, signs, and liver function tests*

Recurrent abnormalities in symptoms, signs and liver function tests have frequently been attributed to the mobilization of a patient who has been at strict bed rest, or to exercise begun too early in the convalescent period (1, 12). The first study

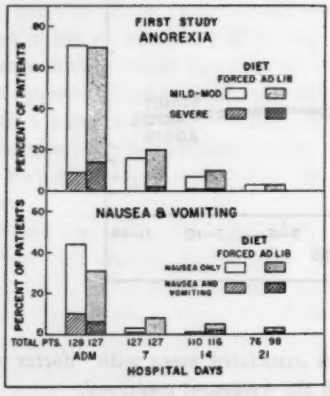

FIG 12. THE EFFECTS OF DIETARY TREATMENT ON THE OCCURRENCE OF ANOREXIA, NAUSEA, AND VOMITING

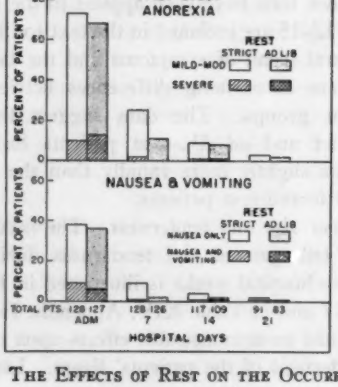

FIG. 13. THE EFFECTS OF REST ON THE OCCURRENCE OF ANOREXIA, NAUSEA, AND VOMITING

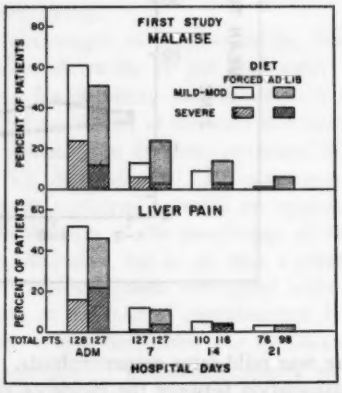

FIG. 14. THE EFFECTS OF DIETARY TREATMENT ON THE OCCURRENCE OF MALAISE AND RIGHT UPPER QUADRANT PAIN

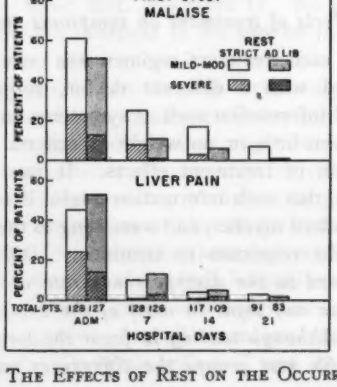

FIG. 15. THE EFFECTS OF REST ON THE OCCURRENCE OF MALAISE AND RIGHT UPPER QUADRANT PAIN

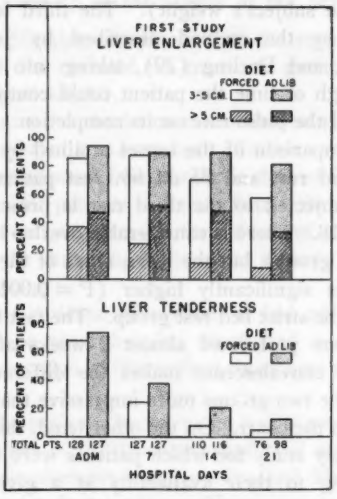

FIG. 16. THE EFFECTS OF DIETARY TREATMENT ON THE OCCURRENCE OF ENLARGEMENT AND TENDERNESS OF THE LIVER

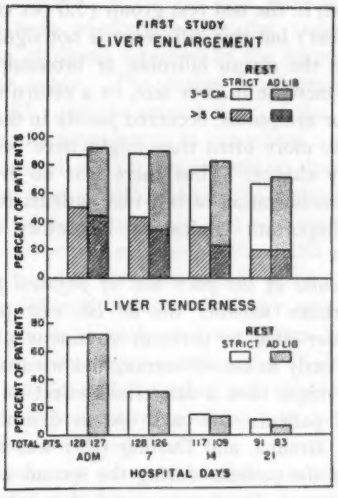

FIG. 17. THE EFFECTS OF REST ON THE OCCURRENCE OF ENLARGEMENT AND TENDERNESS OF THE LIVER

FIGS. 12–17. THE EFFECTS OF THE TREATMENT REGIMENS ON THE OCCURRENCE OF ANOREXIA, NAUSEA, VOMITING, MALAISE, RIGHT UPPER QUADRANT PAIN, ENLARGEMENT AND TENDERNESS OF THE LIVER

offered an opportunity to re-examine these concepts in a controlled manner while at the same time gathering information about the relative ability of patients at strict and *ad lib.* rest to perform exercise during the convalescent period. *In summary, no changes following ambulation could be demonstrated; the ad lib. rest patients were better able to perform physical exertion during the convalescent period, and strenuous exercise resulted in no apparent deleterious effects.*

A. *The effects of ambulation.* For each of the 126 patients on the bed rest wards, the symptoms, liver size and tenderness, and the serum bilirubin and bromsulphalein levels at the start of convalescence and at the end of the first and second weeks of progressive ambulation were recorded. The same data during the equivalent period were tabulated for the 127 patients who were ambulatory *ad lib.* throughout their illness.

Many of the patients still had abnormalities considered by some (3, 5, 28) to be distinct indications for continuation of bed rest. Ambulation was begun as soon as the total serum bilirubin reached 1.5 mg. per 100 ml. instead of the usual 1.0 mg. per 100 ml. (3, 28). The mean serum bilirubins and bromsulphalein retentions at the time of ambulation are presented in Table XV, Appendix II. Forty-eight per cent of the bed rest patients had total serum bilirubins above 1.0 mg. per 100 ml. In 72 per cent the prompt direct bilirubin was above 0.25 mg. per 100 ml., in 6 per cent the bromsulphalein retention in 45 minutes was over 5 per cent.[18] The cephalin flocculation was abnormal in 18 per cent, the thymol flocculation in 40 per cent, the thymol turbidity in 42 per cent and the zinc turbidity in 18 per cent. Fifty-six per cent still had enlarged livers and in 4 per cent tenderness of the liver was still present.

In the strict bed rest group, fluctuations in the incidence of each of these abnormalities occurred after the start of ambulation. These might have been attributed to the change in treatment if they had not occurred with equal frequency in the *ad lib.* rest group whose ambulatory status was unchanged throughout this period. Data illustrating this fact are presented in Table XV, Appendix II.

No patient developed recurrent jaundice during this period. Laboratory "relapses" occurred during the 2 weeks after the start of convalescence

[18] In patients with persistently abnormal bromsulphalein retention, convalescence began, by definition, 4 weeks after their total serum bilirubins were normal.

more often in the bed rest group (5.6 per cent *vs.* 2.4 per cent) but this difference is not significant. A rise in the serum bilirubin or bromsulphalein tests, an increase in liver size, or a return of tenderness or symptoms, occurred jointly in the same patient no more often than might have been expected by chance. Thus there was no evidence that the mobilization of bed rest patients resulted in any important clinical or laboratory abnormalities.

B. *Results of the pack test of physical fitness.* To determine whether the *ad lib.* rest patients were better able to perform strenuous physical exertion early in convalescence, and whether such exertion might have a deleterious effect on either group of patients, the pack test as described by Johnson, Brouha, and Darling (29) was applied to 125 of the patients during the second week of convalescence. The test consisted of stepping onto a bench 20 inches in height every 2 seconds for 5 minutes. Because of the early fear that relapses might be precipitated by such exercise, the test was performed on the first day without a pack, 2 days later with one-half the required pack, and 2 days after that with the full pack (weighing one-third the subject's weight). The third test was scored by the method described by Johnson, Brouha, and Darling (29), taking into account the length of time the patient could continue the test, and the pulse rate on its completion.

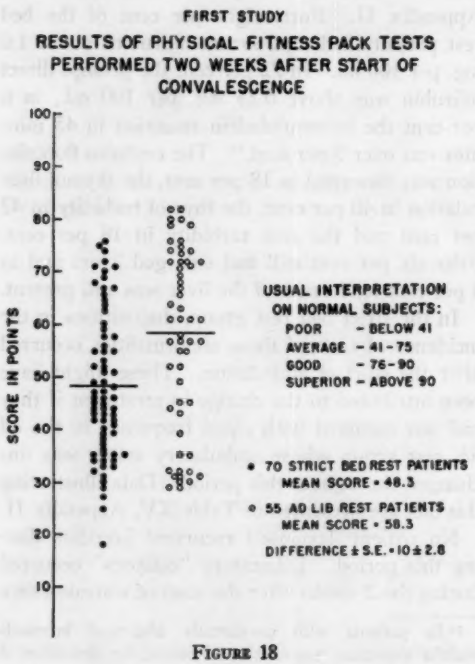

FIGURE 18

A comparison of the scores attained by the 70 strict bed rest and 55 *ad lib.* rest patients who were subjected to the third test is presented in Figure 18. There is considerable overlap between the two groups, but the mean score of the *ad lib.* group is significantly higher ($P = 0.001$) than that of the strict bed rest group. The fact that the tests were performed almost 2 weeks after the start of convalescence makes the difference between the two groups more impressive than it appears on the chart. On the other hand, this was a subsidiary study for which patients were selected according to their availability at a given time rather than at random, and the results, while of interest, may not be as reproducible as implied by the significance test.

A compilation of the results of serum bilirubin and bromsulphalein tests and physical examination performed on the day after the third test revealed no difference between the two groups of patients.

C. *The effects of strenuous physical reconditioning.* Two hundred and two patients were transferred to the Physical Reconditioning Center at Nara (subsequently referred to as the P.R.C.) before their return to active duty. The remaining patients, for various administrative reasons, were sent directly to duty on discharge from the hospital. The patients were discharged from the hospital to the P.R.C. from the 12th to the 79th day after convalescence, the average being 26 days. The means and ranges of total and prompt direct serum bilirubin values on discharge to the P.R.C. are presented in Table XVI, Appendix II. A physical and laboratory check-up was usually performed once during the 10 to 14 days the patients were at the P.R.C. Only two patients had asymptomatic bromsulphalein relapses commencing during physical reconditioning. Although the system of following patients at the P.R.C. in the first study was not well organized and there were no control groups of patients, there was no evidence that strenuous exercise during this stage of convalescence had any deleterious effects.

3.11 *Summary of the results of the first study*

1. Verification of adherence to the regimens of rest and diet revealed the treatment groups to be distinctly dissimilar.

2. Numerous checks on the method of randomization revealed it to have been effective.

3. The removal from the basic data of 7 of the original 260 patients did not change the results.

4. Several analyses of the effects of diet and rest on the duration of the acute illness revealed the following:

a) The patients allowed out of bed *ad lib.* but confined to their hospital ward had a slightly shorter duration of illness than those kept at strict bed rest.

b) The patients forced to eat a diet containing a minimum of 3000 calories and 150 grams of protein supplemented with choline and Multivitamin capsules, had an illness 22 per cent or 6 days shorter in duration than those who were allowed to eat the regular hospital diet *ad lib.* This difference was highly significant.

c) There was no interaction between the effects of diet and rest.

5. Asymptomatic bromsulphalein relapses occurred in 7 per cent of the patients during the observation period and were equally distributed among the treatment groups.

6. There was a significantly more rapid fall of the mean serum bilirubins in the forced-diet group than in the *ad lib.* group. There was a tendency for the flocculation and turbidity tests to be more abnormal at the end of the acute illness in the forced-diet group, but these tests were the same in both groups at the time of discharge.

7. The various treatments had no striking effects on the patients' symptoms and signs.

8. Ambulation of the strict bed rest patients while they still had some abnormalities in physical signs or liver function tests was not followed by demonstrable deleterious effects.

PART FOUR

SECOND STUDY: THE EFFECTS OF VARIOUS DIETARY COMPONENTS

4.1 *Design*

The purpose of the second study was to determine which of the dietary factors—total calories, protein content, or choline and vitamin supplements—were responsible for the reduction in duration of illness achieved by the forced diet in the first study. The design is diagrammed in Figure 23. On the second floor of the hospital, the patients were forced to eat within a range of ± 10 per cent of 4000 calories and on the 4th floor ± 10 per cent of 3000 calories. On the wards at one end of the hospital the diet was composed of 19 per cent protein calories (140 and 190 gm. ± 10 per cent) and at the other end 11 per cent protein calories (80 and 110 gm. ± 10 per cent). Half of the patients on each ward were given 3.9 grams of choline dihydrogen citrate and 6 Multivitamin capsules daily, and the other half received placebos.[19] All patients were allowed to be up and about their wards *ad lib.*

The primary purpose of this treatment plan was to determine if an abundance of each dietary factor had any advantage over an adequate or "normal" amount. Although there was some retrospective evidence from the first study suggesting that lower than "normal" intakes in the acute phase of the disease were usually followed by a prolonged illness, it was considered unwise to investigate this effect in the second study because definite restriction of intake below "normal" in one group of patients would have been required. Since such restriction might well have been harmful, and certainly difficult to achieve, it was decided to accept the results of the first study as indicating that forcing to a "normal" level of intake is advantageous in the anorectic phase of the disease, and attempt to determine in the second study whether high levels of calories, protein, or choline and vitamins have any advantage over "normal" amounts.

The criteria for admission were the same as in the first study, and patients were assigned to treatments in blocks. Although there were eight treatment groups, it seemed desirable to restrict the size of each block to four patients in hopes that such a small block would be more homogeneous on admission than would a block of eight. Each complete set of eight treatments was therefore composed of two kinds of blocks, as follows:

[19] For supplying the choline dihydrogen citrate tablets and their placebos the authors wish to thank Dr. George R. Hazel, Abbott Laboratories, North Chicago, Ill.

Type A

High calories, high protein, supplements (CPS)
"Normal" calories, high protein, placebos (cPs)
High calories, "normal" protein, placebos (Cps)
"Normal" calories, "normal" protein, supplements (cpS)

Type B

High calories, high protein, placebos (CPs)
"Normal" calories, high protein, supplements (cPS)
High calories, "normal" protein, supplements (CpS)
"Normal" calories, "normal" protein, placebos (cps)

It was necessary to randomize the chronological order in which the two kinds of blocks were filled as well as the order of assigning four consecutive patients to the treatments in each block. These assignments were made with the use of a table of random numbers before the study began. *Two hundred patients were admitted to the study* in 50 blocks, 25 of each type.

The same four wards were used in the second study as in the first. An opportunity was afforded to control partially the unknown effects of the physician on the course of his patients' hepatitis by assigning the doctors to different wards. Those who cared for the second floor, forced-fed patients in the first study moved to the fourth floor where the diets contained fewer calories and less protein than the corresponding second floor diets, and vice versa.

4.2 *Rest regimen*

All patients in this study were allowed to be out of bed ad lib. except for a 1 hour rest period after each meal. They were confined to their hospital wards until the start of convalescence. Recording of the amount of time each patient spent in bed was performed as on the *ad lib.* rest wards in the first study, and the results were similar.

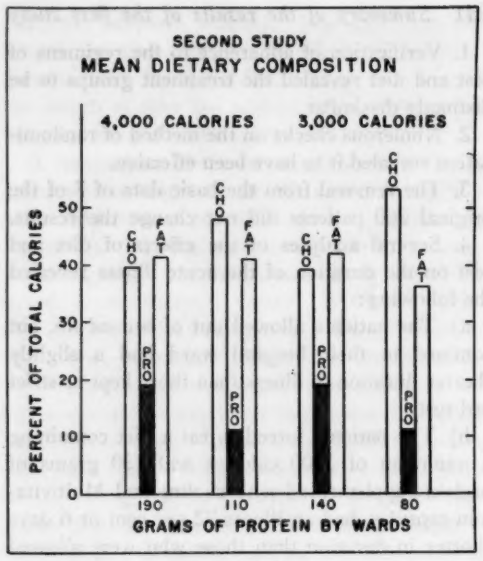

FIGURE 19

4.3 *Dietary regimens*

The content of each of the four basic diets is presented in Table X and Figure 19. It is apparent that as percentage of protein was decreased each caloric level was maintained by adding carbohydrate, and that at a constant percentage of protein calories the total calories were decreased by reducing the amounts of all three dietary substances. In terms of protein content in grams there were four different diets; in terms of per-

TABLE X

Second study: Contents of a typical day's diet on each of the four wards *

	Calories	CHO gm.	Pro gm.	Fat gm.	Choline base† gm.	Methionine gm.	Vit. A I.U.	Thiamin mg.	Riboflavin mg.	Niacin mg.	Vit. C mg.
Ward 23 (CP)	3,992	357	191	200	1.73	4.7	15,000	2.5	5.4	28.8	105
Ward 43 (cP)	2,986	296	140	138	0.88	3.4	13,000	2.1	4.0	24.2	112
Ward 21 (Cp)	4,010	446	111	198	0.70	2.5	14,000	1.9	3.6	19.5	115
Ward 41 (cp)	3,002	406	79	118	0.38	1.8	12,000	1.8	2.1	20.3	201

* Supplements were administered to half the patients on each ward as follows:

Vitamin A	15,000 USP units	Riboflavin	9 mg.
Vitamin D	1,200 USP units	Vitamin C	225 mg.
Thiamin	6 mg.	Nicotinamide	60 mg.
		Choline base†	1.6 gm.

† To convert to choline chloride multiply by 1.3 and to choline dihydrogen citrate by 2.4.

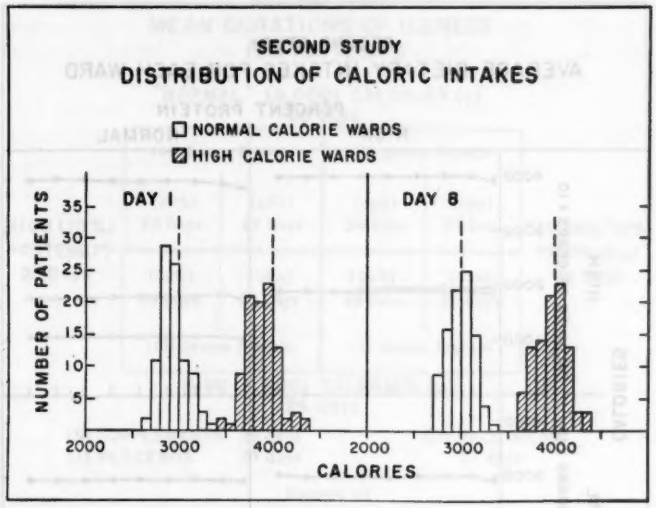

FIGURE 20

centage of protein there were two levels. The fat content varied with the calories but when averaged over calories was essentially the same at the two percentages of protein levels. In the second study all food portions were weighed individually and the smaller number of patients on the wards allowed for closer supervision of each patient's intake.

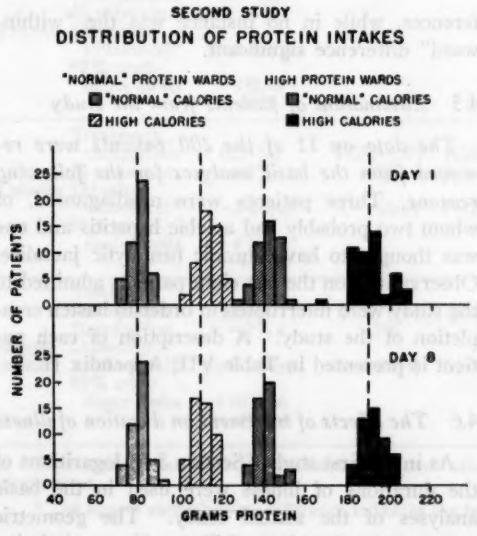

FIGURE 21

The narrow distribution of caloric and protein intakes on the first and eighth hospital days are illustrated in Figures 20 and 21. This is in contrast to the wide range of intakes in both the forced and *ad lib.* dietary groups of the first study (see Figures 6 and 7, Section 3.3). Also different from the first study are the dietary trends presented in Figure 22. Except for a small rise between days 1 and 4 the mean dietary intakes in this study are constant throughout. Here again it can be seen that the fat content of the high and "normal" protein diets is the same.

To maintain the dietary intakes within the prescribed limits, 22 patients had to be tube-fed in the second study—15 for 1 day only, 5 for 2 days, 1 for 3 days, and 1 for 4 days. Seventeen of the tube-fed patients were on the 4000-calorie ward and 5 on the 3000-calorie ward.

4.4 *Effectiveness of the randomization*

Analysis of numerous variables revealed the randomization to have been effective. Tests for the effectiveness were carried out in a manner similar to that used in the first study (Section 3.4). Because of the smaller number of patients assigned to each group, a single "chi-square" test could not be used to test for differences in the percentage distribution of a qualitative variable over the eight treatment groups. Therefore, two

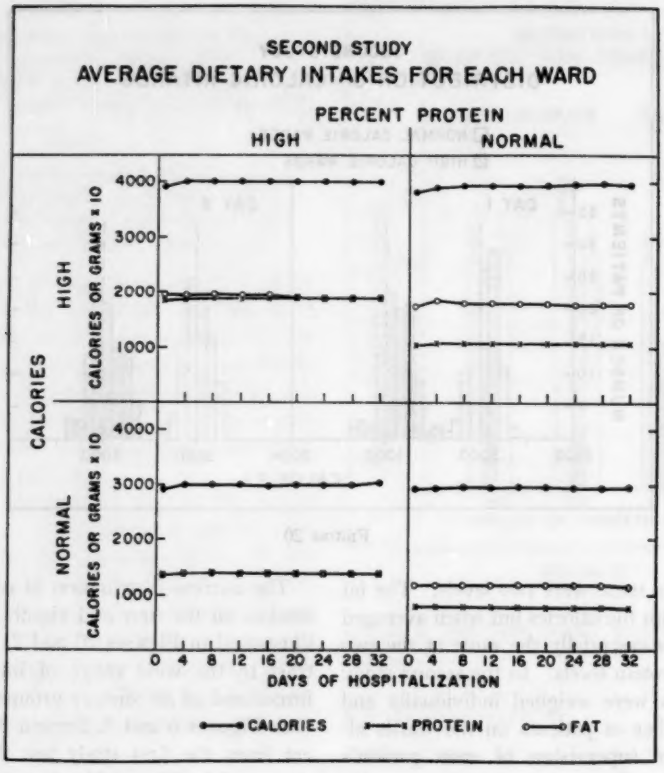

FIGURE 22

"chi-square" tests were used for each of 21 qualitative items—one for a comparison of differences among the four wards and the other for within-ward comparison of the group of patients given supplements (S) with those given placebos (s). Differences among the means of the eight treatment groups were tested for significance in an "analysis of variance" for each of 15 quantitative variables. In the case of those variables which showed significant differences, a separate "within-ward" comparison was also made between the means of the "supplement" and "placebo" groups.

Details of the significance tests applied to 36 variables are presented in Table VI, Appendix II. Among the 14 variables thought unlikely to be influenced by "doctor effects," only one, race, showed significant differences among the treatment groups. On the other hand, among the variables thought to be subject to "doctor effects," 13 out of 22 showed significant "between-ward" differences, while in no instance was the "within-ward" difference significant.

4.5 *Elimination of patients from the study*

The data on 11 of the 200 patients were removed from the basic analyses for the following reasons: Three patients were misdiagnosed, of whom two probably had amebic hepatitis and one was thought to have chronic hemolytic jaundice. Observations on the last eight patients admitted to the study were interrupted in order to hasten completion of the study. A description of each patient is presented in Table VII, Appendix II.

4.6 *The effects of treatment on duration of illness*

As in the first study (Section 3.6) logarithms of the durations of illness were used in the basic analyses of the second study. The geometric means of the durations of illness (from admission

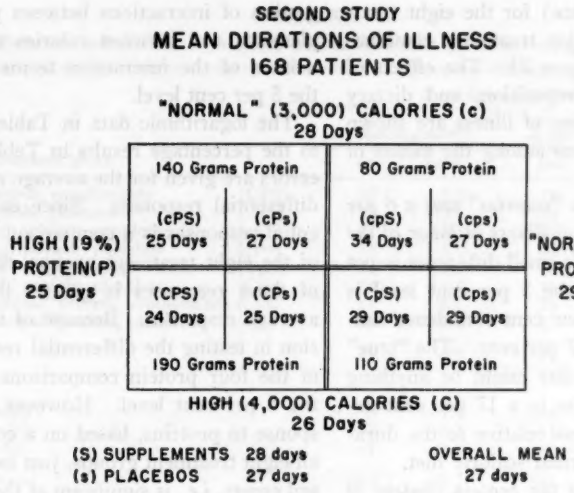

FIGURE 23

Since basic calculations were performed in logarithms, the means in this figure are geometric.

TABLE XI

Second study: Responses to treatment: Percentage reduction in duration of illness—168 patients

Factor		Average response	Response with: Protein: High	Protein: "Normal"	Calories: High	Calories: "Normal"	Supplements: Added	Supplements: Placebos
Protein	Treatment comparison	(P)–(p)			(CP)–(Cp)	(cP)–(cp)	(PS)–(pS)	(Ps)–(ps)
	% reduction	−14%			−13%	−14%	−21%	−6%
	(Absolute difference)*	(−4 days)			(−4 days)	(−4 days)	(−7 days)	(−2 days)
	95% confidence limits	−2% to −24%						
Calories	Treatment comparison	(C)–(c)	(CP)–(cP)	(Cp)–(cp)			(CS)–(cS)	(Cs)–(cs)
	% reduction	−6%	−5%	−6%			−9%	−2%
	(Absolute difference)*	(−2 days)	(−1 day)	(−2 days)			(−3 days)	(−0.5 day)
	95% confidence limits	+8% to −17%						
Supplements	Treatment comparison	(S)–(s)	(PS)–(Ps)	(pS)–(ps)	(CS)–(Cs)	(cS)–(cs)		
	% reduction	−2%	−7%	−10%	−2%	−6%		
	(Absolute difference)*	(+0.5 day)	(−2 days)	(+3 days)	(−0.5 day)	(+2 days)		
	95% confidence limits	+10% to −14%						

Treatment levels: C—high calories; c—"normal" calories; P—high protein; p—"normal" protein; S—supplements; s—placebos.

* The sign of each absolute difference indicates the favorable treatment.

to the start of convalescence) for the eight treatment groups and the major treatment combinations are presented in Figure 23. The effects of caloric intake, protein composition, and dietary supplements on the duration of illness are shown in Table XI as comparisons among the means of Figure 23.

The average response to "calories" was a 6 per cent reduction in duration of illness in favor of the high-calorie diet. That this small difference is not statistically significant at the 5 per cent level is demonstrated by the 95 per cent confidence limits of + 8 per cent to − 17 per cent. The "true" effect of the high-calorie diet might be anything from an 8 per cent increase to a 17 per cent decrease in duration of illness relative to the duration produced by the "normal"-calorie diet.

The average response to the protein content of the diet was a 14 per cent reduction in duration of illness achieved by the high-protein diet with 95 per cent confidence limits of − 2 per cent to − 24 per cent. This result is significant at the 5 per cent level although the "true" effect may lie anywhere on the rather wide confidence interval.

The average response to supplements was negligible, a 2 per cent reduction favoring the placebos with 95 per cent confidence limits of + 10 per cent to − 14 per cent. The differential responses to each treatment factor at the two levels of each of the other factors are also shown in Table XI. Although there is a qualitative suggestion of interactions between protein and supplements and between calories and supplements, neither of the interaction terms is significant at the 5 per cent level.

The logarithmic data in Table XII correspond to the percentage results in Table XI. Standard errors are given for the average responses and the differential responses. Since each of the differential responses is a comparison among only four of the eight treatment groups, the standard error of these responses is greater than that for the average responses. Because of this loss of precision in testing the differential responses, only one of the four protein comparisons is significant at the 5 per cent level. However, the average response to proteins, based on a comparison among all eight treatment groups, just exceeds two standard errors, *i.e.*, is significant at the 5 per cent level. None of the differential responses to calories or supplements approach significance.

Several subsidiary analyses were also done, comparable to those used in the first study. The results are shown in Tables XIII and XIV. The mean values in these tables are arithmetic means. The results are similar to those of the basic analyses, irrespective of the definition of treatment effect or the manner of handling the problem of "eliminated patients." Note, however, that in the analyses based on the first normal bromsulphalein retention the average response to protein is not significant at the 5 per cent level.

TABLE XII

Second study: Responses to treatment: Logarithms of ratios of geometric mean durations—168 patients

Factor		Average response	Response with: Protein: High	Protein: "Normal"	Calories: High	Calories: "Normal"	Supplements: Added	Supplements: Placebos
Protein	Treatment comparison	(P)/(p)			(CP)/(Cp)	(cP)/(cp)	(PS)/(pS)	(Ps)/(ps)
	Logarithm of ratio	−0.0647			−0.0625	−0.0669	−0.1042	−0.0252
Calories	Treatment comparison	(C)/(c)	(CP)/(cP)	(Cp)/(cp)			(CS)/(cS)	(Cs)/(cs)
	Logarithm of ratio	−0.0247	−0.0225	−0.0269			−0.0420	−0.0074
Supplements	Treatment comparison	(S)/(s)	(PS)/(Ps)	(pS)/(ps)	(CS)/(Cs)	(cS)/(cs)		
	Logarithm of ratio	+0.0088	−0.0307	+0.0483	−0.0085	+0.0261		
Standard error of response		±0.0293			±0.0414			

TABLE XIII

Second study: Responses to treatment: Influence of various definitions of the duration of illness—168 patients

Treatment group		Mean duration of illness from admission to: Convalescence* (days)	Convalescence* (weeks)	First normal TSB (days)	First normal BSP (days)	Last abnormal† TSB or BSP (days)
High calories, high protein, supplements	(CPS)	27	3.3	20	29	32
High calories, high protein, placebos	(CPs)	28	3.4	22	28	29
"Normal" calories, high protein, supplements	(cPS)	27	3.3	21	30	36
"Normal" calories, high protein, placebos	(cPs)	32	4.0	24	32	39
High calories, "normal" protein, supplements	(CpS)	32	4.0	25	35	38
High calories, "normal" protein, placebos	(Cps)	34	4.3	26	36	41
"Normal" calories, "normal" protein, supplements	(cpS)	37	4.7	26	37	40
"Normal" calories, "normal" protein, placebos	(cps)	33	4.1	27	33	40
Overall mean		31	3.9	24	33	37
Average response to:						
Protein	(P)–(p)	−6	−0.8	−4	−5	−6
Calories	(C)–(c)	−2	−0.2	−1	−1	−3
Supplements	(S)–(s)	−1	−0.1	−2	+1	−1
Standard error of response		±2.2	±0.3	±1.5	±3.0	±2.9

* By prior definition the acute illness ended and convalescence started as soon as the total serum bilirubin had been 1.5 mg./100 ml. or less twice and the bromsulphalein retention 5% or under once, and could be prolonged by a persistently elevated bromsulphalein test by no more than 4 weeks after 2 normal serum bilirubins.

† Includes all "relapses" during convalescence in hospital and at Physical Reconditioning Center.

TABLE XIV

Second study: Treatment groups compared according to various methods of handling "eliminated patients": Mean duration of illness from admission to convalescence in days

Treatment group		Replicates containing "eliminated patients" removed	"Eliminated patients" only removed	All patients included
High calories, high protein, supplements	(CPS)	27	27	28
High calories, high protein, placebos	(CPs)	28	28	28
"Normal" calories, high protein, supplements	(cPS)	27	29	29
"Normal" calories, high protein, placebos	(cPs)	32	31	32
High calories, "normal" protein, supplements	(CpS)	32	32	33
High calories, "normal" protein, placebos	(Cps)	34	36	36
"Normal" calories, "normal" protein, supplements	(cpS)	37	37	39
"Normal" calories, "normal" protein, placebos	(cps)	33	34	33
Average response to:				
Protein	(P)–(p)	−6	−6	−6
Calories	(C)–(c)	−2	−2	−2
Supplements	(S)–(s)	−1	−1	0
Standard error of response		±2.2	±2.0	±2.0
Number of patients		168	189	192

4.7 *Laboratory "relapses"*

The distribution of "relapses" among dietary regimens is presented in Table XV. None of the differences are significant. As in the first study, recurrent jaundice was never observed during convalescence.

4.8 *Other effects of treatment on laboratory tests*

The mean serum bilirubins and bromsulphalein retentions fell more promptly in the high-protein group. No dietary effects on the other laboratory tests were demonstrated.

A. *Trends of mean serum bilirubin and brom-*

TABLE XV

Second study: Occurrence of "relapses" by treatment regimen

	Number of patients	Partial "relapse"					Complete "relapse"				
		TSB* No.	BSP† No.	Total No.	Total %	P	TSB* No.	BSP† No.	Total No.	Total %	P
High protein	94	9	6	15	16		0	4	4	4	
"Normal" protein	95	8	13	21	22	.3	1	3	4	4	—
High calorie	95	11	5	16	17		1	4	5	5	
"Normal" calorie	94	6	14	20	21	.4	0	3	3	3	.5
Supplements	95	9	9	18	19		0	1	1	1	
Placebos	94	8	10	18	19	—	1	6	7	7	.06

* Total serum bilirubin only abnormality.
† Bromsulphalein retention only abnormality.

sulphalein retention. As in the first study, there were distinct differences among the dietary regimens in the curves of the mean prompt direct and total serum bilirubins during the first 2 hospital weeks (see Figure 24). In the protein comparison the difference was striking in favor of the high-protein diets and was confirmed statistically by comparing the mean changes between admission and the 11 to 14-day period (Table XVI). In addition, a significantly greater percentage of patients on the "normal"-protein diets had rises in serum bilirubin after admission (Table VIII, Appendix II). There were no differences between the high- and "normal"-calorie groups by these three criteria. Those receiving choline and vitamin supplements had slightly more rapid falls than the patients receiving placebos. This was not confirmed by significant differences between the mean falls from admission to the 11 to 14-day period, but significantly more patients receiving placebos did have rises in both total and prompt direct serum bilirubins.

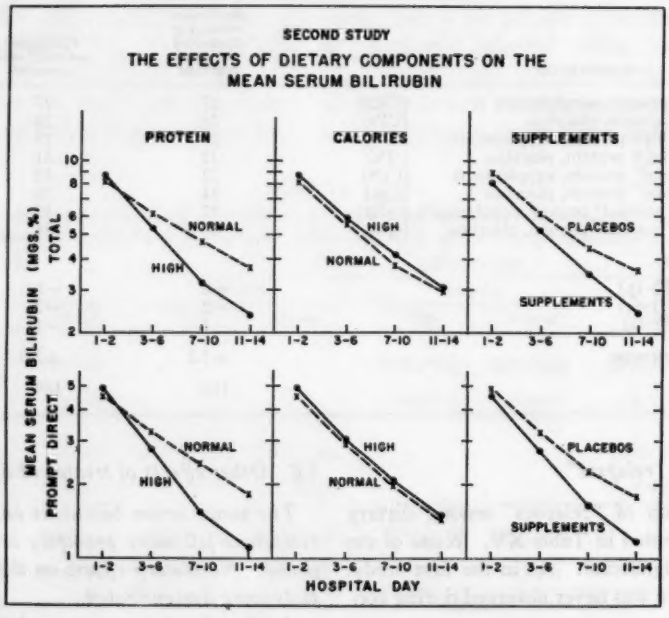

FIGURE 24

TABLE XVI

Second study: Mean fall in serum bilirubin during the first 11 to 14 days of each treatment regimen

	Protein			Calories			Supplements		
	High	"Normal"	P	High	"Normal"	P	Added	Placebos	P
Number of patients	93	92		92	93		94	91	
Total serum bilirubin									
Admission value	8.56	8.08		8.60	8.05		8.19	8.44	
Mean fall	6.37	4.63	.01	5.76	5.25	.5	5.80	5.20	.4
S.D.	4.68	4.54		4.20	5.16		4.83	4.44	
Prompt direct serum bilirubin									
Admission value	4.58	4.78		4.90	4.46		4.55	4.81	
Mean fall	3.78	2.80	.02	3.50	3.08	.3	3.43	3.14	.5
S.D.	2.87	2.64		2.56	3.00		2.80	2.80	

In the second study the bromsulphalein test was performed on admission and at weekly intervals after the total serum bilirubin reached 1.5 mg. per 100 ml. or less. The data in Table XVII show that bromsulphalein excretion also tended to improve more rapidly on the high-protein diet.

B. *Thymol turbidity, zinc turbidity, cephalin flocculation, and thymol flocculation.* Trends of the mean thymol and zinc turbidity values during the first 2 weeks did not show any differences among the dietary regimens. Comparisons of the number of patients in whom tests rose in the first 2 weeks likewise showed no difference. There were also no differences among the treatment groups in the mean values for the thymol and zinc turbidity tests on admission, at convalescence, and on discharge to duty (Table IX, Appendix II).

The percentages of patients with values above the 99 per cent upper limit of normality at the time of admission, at any time during the hospital course, at convalescence and on discharge to duty are presented in Table X, Appendix II. With the exception of a higher percentage of abnormal cephalin flocculations at the time of convalescence in the "normal"-protein group when compared to the high-protein group ($P = 0.05$), there were no differences. This one significant difference was the only one encountered out of 75 significance tests performed in this group of analyses and therefore is probably a chance occurrence.

C. *Serum cholinesterase.* Serum cholinesterase values were abnormally low on admission. The mean for all patients in the second study was 0.64 pH per hr., which is significantly different from 0.80, the mean for the 279 "normal" soldiers (S.E. of the difference = 0.02). During the hospital course the serum cholinesterase values showed a general trend to higher levels which were comparable in the several calorie and protein groups (Table XII, Appendix II).

TABLE XVII

Second study: Mean bromsulphalein retention (per cent in 45 minutes) on admission and during the two weeks after total serum bilirubin became normal

	Protein			Calories			Supplements		
	High	"Normal"	P	High	"Normal"	P	Added	Placebos	P
Number of patients	94	95		95	94		95	94	
Admission value	45.9	45.8	—	46.4	45.3	.5	46.0	44.7	.5
S.D.	13.2	13.0		12.8	13.1		13.6	15.8	
First week after normal TSB	6.6	8.3	.05	7.5	7.4	.5	7.9	7.0	.3
S.D.	5.6	5.9		6.1	5.5		6.2	5.4	
Second week after normal TSB	4.7	5.8	.1	5.0	5.6	.3	5.3	5.2	—
S.D.	3.8	5.2		4.5	4.5		4.3	4.8	

4.9 *Effects of treatment on symptoms and signs*

As discussed previously (Section 3.6) the influence of "doctor effects" on the data obtained for symptoms, liver size and tenderness, splenomegaly and spider angiomata was so great that attempts to interpret differences in these factors in terms of treatment effects seem unwarranted. Data from the second study are presented in Tables XIII and XIV, Appendix II. There are no striking differences.

The weight changes during the first 3 hospital weeks are presented in Table XIV, Appendix II. The patients on the high-calorie diets gained more weight than the "normal"-calorie group during the first 2 weeks only.

4.10 *Summary of the results of the second study*

1. Carefully collected dietary data demonstrated strict adherence to the prescribed diets.

2. Numerous checks on the method of randomization showed it to have been effective.

3. Several analyses of the effects of caloric intake, protein composition of the diet, and supplementary choline and vitamins on the duration of acute infectious hepatitis revealed the following:

a) Patients fed a high-protein diet (19 per cent protein calories) throughout their illness had a mean duration of illness 14 per cent shorter than the duration of patients fed a "normal"-protein diet (11 per cent protein calories). The probability that a difference of this size or greater might occur by chance is less than one in twenty.

b) Patients fed a high-calorie diet (4000 cal.) did not have a significantly shorter duration of illness than those fed a "normal"-calorie diet (3000 cal).

c) Patients given supplementary choline and a Multivitamin preparation did not have a shorter duration of illness than patients who were given placebos.

d) There were no demonstrable interactions among the three dietary factors.

4. Asymptomatic bromsulphalein and serum bilirubin relapses occurred in 4 per cent of the patients without any significant differences among the treatment groups.

5. There were significantly more rapid falls of both total and prompt direct serum bilirubins in the high-protein groups than in the "normal"-protein groups. Questionably more rapid falls were observed in patients receiving supplements as compared with those given placebos.

PART FIVE

THIRD STUDY:[20] THE EFFECTS OF STRENUOUS EXERCISE DURING CONVALESCENCE

5.1 *Design*

The purpose of the third study was to determine if the duration of hospitalization could safely be shortened by eliminating the 1 to 3 weeks usually necessary for patients to recuperate from the effects of strict bed rest. Half of the second study patients, all of whom had been at *ad lib.* rest, were accordingly started on strenuous physical reconditioning as soon as their total serum bilirubins reached 1.5 mg. per 100 ml. or less, and their bromsulphalein retentions were 5 per cent or less. The other half were observed in the hospital as controls. *The patients in the early exercise group developed significantly more minor abnormalities, but these were considered of little clinical importance because they disappeared while exercise was continued.*

Following admission to one of the eight treatment groups of the second study by the procedure outlined in Section 4.1, half of the patients in each dietary group were randomly assigned to each of the two exercise groups. The randomization was restricted to pairs in order to keep the two groups equal in size as the second study progressed.

All patients in the second study were transferred to the Physical Reconditioning Center (P.R.C.) at Nara, Japan, in either an "early" or a "late" group. Elective operations and dental work were not done until after the patients had completed their reconditioning program. Transfer was requested for patients in the "early" group as soon as they fulfilled the criteria for the start of convalescence (total serum bilirubin below 1.5 mg. per 100 ml. and bromsulphalein retention in 45 minutes below 5 per cent). Various administrative procedures then resulted in a delay of 1 to 7 days before actual transfer took place, the average interval being 3 days for the "early"

[20] For his help in the performance of this study the authors wish to thank Hunter A. Soper, 1st Lt., M.C., U.S.A.R.

group. Transfer was requested for the "late" group on the 8th day of convalescence and these patients were actually transferred, on the average, on the 12th day. As shown in Table XVI, Appendix II, the mean serum bilirubin levels at the time of transfer of the "early" group are significantly higher than those of the "late" group, and the latter are significantly higher than the values of the first study patients at the time of transfer. The mean serum bilirubin levels of all three groups of patients, at the start of exercise, were significantly higher than normal.

At the P.R.C. the hepatitis patients joined in the active reconditioning program designed for all patients convalescing from hospitalization in Southwest Japan. The mornings were spent attending classes and movies, engaging in various types of graduated calisthenics and reconditioning exercises, and taking part in short marches and drills in the camp area. On Mondays the subjects took a 7.5-mile, 3-hour hike over flat terrain. On Wednesdays they took a 5-mile march in 2 hours over rougher terrain, and on Fridays they were assigned a 9.5-mile road march over rugged mountain terrain to be completed in 3½ hours. On Tuesdays, Thursdays, and Saturdays, they participated in organized athletics such as basketball, volley ball, soccer, and soft ball. There were no restrictions on week-end and evening passes. Although patients were admonished not to drink alcoholic beverages, no action was taken if they chose to disregard the warnings.

Although the reconditioning program was designed to allow increasing activity from the beginning to the end of each week, many of the patients were transferred at the middle or end of the week, and thus engaged in the more strenuous exercise immediately. Patients in the "early" group were kept at the P.R.C. for a total of 2 weeks so that they might be under observation the same length of time as the "late" group transferred approximately a week later. Between convalescence and transfer to the P.R.C. those in the "late" group were allowed *ad lib.* activity throughout the hospital.

Patients were seen twice a week by one of the authors or by a doctor of the P.R.C. staff. A brief history and physical examination were performed and the same observations made as described in Section 2.4. The bromsulphalein test was done weekly and blood for other liver function tests was drawn twice a week. The tests were performed in the same laboratory (Kyoto) as were those of the first and second studies. The resulting data were analyzed with respect to three time periods as follows:

Period I: The week immediately preceding convalescence, including three semi-weekly days on which clinical or laboratory observations were made.

Period II: The first week of exercise at the P.R.C. for the "early" group and the last week in the hospital before transfer for the "late" group. For most patients there were two observations in this period.

Period III: The second week of strenuous exercise for the "early" group and the first week at the P.R.C. for the "late" group.

5.2 *Elimination of patients from the study*

Although 192 patients were included in the original randomization as performed on admission to the second study, 10 patients have been excluded from the third study analyses for the following reasons: Three patients (one in the "early" group, two in the "late") were demonstrated after admission not to have infectious hepatitis. Three patients still had abnormal bromsulphalein tests 120 days after admission (all in the "early" group) and were sent to limited duty without physical reconditioning. Four patients in the "early" exercise group, because of an administrative oversight, were transferred more than 1 week after convalescence. In addition, in the "late" group, the transfers of three patients were delayed more than 1 week by oversight and four patients were transferred late because they developed partial or complete relapses in the hospital. These seven patients are included in the analyses of the period II data but are left out of the over-all laboratory curves and period III analyses. Thus, in comparisons involving periods I and II as defined above, there were 88 patients in the "early" group and 94 in the "late." In the period III analyses there were 88 in the "early" group and 87 in the "late."

5.3 *Effectiveness of the randomization*

The randomization was not as effective as in the first and second studies. This is probably at-

tributable both to the original restriction of the randomization to pairs and to the larger number of patients removed from the study after assignment to a treatment regimen. For 3 of the 25 variables examined (see Table VI, Appendix II) there was a significant difference between the two groups. The "early" group had a higher mean bromsulphalein retention and fewer positive thymol flocculations on admission than the "late" group. Although not significant, the admission total serum bilirubin was also higher in the "early" group (8.95 mg. per 100 ml. compared wtih 7.96 mg. per 100 ml.). These results suggest that, by chance, the "early" group included sicker patients at the time of admission to the second study, and this impression is confirmed by the fact that their average duration of illness was 34 days as compared with 28 days for the "late" group ($P = 0.02$). However, as illustrated in Figures 25 and 26, the mean serum bilirubins and bromsulphalein retentions were essentially the same for the two groups at the start of the convalescent period.

5.4 *Effects of physical reconditioning on clinical and laboratory findings*

It was the opinion of the medical officers in charge of the reconditioning program that the hepatitis patients had relatively fewer complaints than those patients convalescing from battle wounds, epidemic hemorrhagic fever, or other illnesses. Nevertheless, there were some differences among the hepatitis patients with respect to the distributions of changes in symptoms and liver size, as shown in Table XVIII. During period II symptoms appeared more often in the "early" group undergoing exercise then in the "late" patients who remained in the hospital. Of the 10 patients in the "early" group who developed symptoms during their first week at the P.R.C., 3 had mild anorexia only, 2 had mild malaise when active but were asymptomatic at rest, 4 complained of mild epigastric distress on exertion, and 1 had both mild pain and malaise. The proportion of patients in whom previous symptoms disappeared

TABLE XVIII

Third study: Changes in symptoms, liver size (by 2 cm. or more), total serum bilirubin (by 0.25 mg. per 100 ml.), prompt direct serum bilirubin (by 0.05 mg. per 100 ml.), and bromsulphalein retention (by 1 per cent or more)

	Period II*			Period III†		
	"Early"	"Late"	P	"Early"	"Late"	P
Number of patients	88	94		88	87	
Symptoms						
Appeared	10	1		14	10	
No change	66	79	.02	68	74	.4
Disappeared	12	14		6	3	
Liver size						
Increase	7	2		7	13	
No change	48	67	.05	68	52	.06
Decrease	33	25		13	22	
Total serum bilirubin						
Increase	19	2		3	13	
No change	58	62	.001	57	59	.01
Decrease	11	30		28	15	
Prompt direct serum bilirubin						
Increase	4	2		3	11	
No change	28	15	.05	36	43	.001
Decrease	56	77		49	33	
Bromsulphalein retention						
Increase	18	4		22	32	
No change	27	35	.01	24	30	.05
Decrease	43	55		42	25	

* At the end of period II the "early" group had exercised for one week and the "late" group had remained in the hospital as controls.

† At the end of period III the "early" group had exercised for two weeks and the "late" group had exercised for one week.

during period II was about the same in the two groups. During period III in which both groups were at the P.R.C., the pattern of changes in symptoms was about the same for the two groups and all new symptoms were benign.

Table XVIII also shows a significant difference between the distributions of change in liver size for the two groups during period II. There were more decreases as well as increases in liver size in the "early" group with fewer patients showing no change. In only one of the seven patients in the "early" group who had increases in liver size was the increase greater than 2 cm. In period III the differences were not significant although the "late" group, then in their first week of reconditioning, tended to show more fluctuations in liver size. *No patient in either group developed tenderness of the liver during reconditioning.*

It seems likely that some of the changes in symptoms and liver size after transfer to the P.R.C. reflect differences among observers, but no data are available to test this assumption.

Complete laboratory "relapses," defined by the consecutive occurrence of at least two abnormal bromsulphalein or total serum bilirubin tests, were observed in 4 per cent of the patients. This rate was similar to that found in the first study (7 per cent) in which the patients started physical reconditioning an average of 19 days later in their course. No patient in either the "early" or "late" group developed recurrent hepatitis with jaundice during or shortly after the period of reconditioning. Two patients in the "early" group and five in the "late" group had asymptomatic bromsulphalein "relapses," but two of the latter occurred before the start of strenuous exercise. Sixteen per cent of the "early" group and only 4 per cent of the "late" had one total serum bilirubin determination above normal after the start of convalescence ($P = 0.02$). The incidence of partial bromsulphalein "relapses" was approximately the same for each group, 9 per cent and 12 per cent, respectively.

Graphs of the changes in mean total and prompt direct serum bilirubin and bromsulphalein retention are shown in Figures 25 and 26, in which the two vertical lines separate the three time periods defined in Section 5.1. There was a distinct

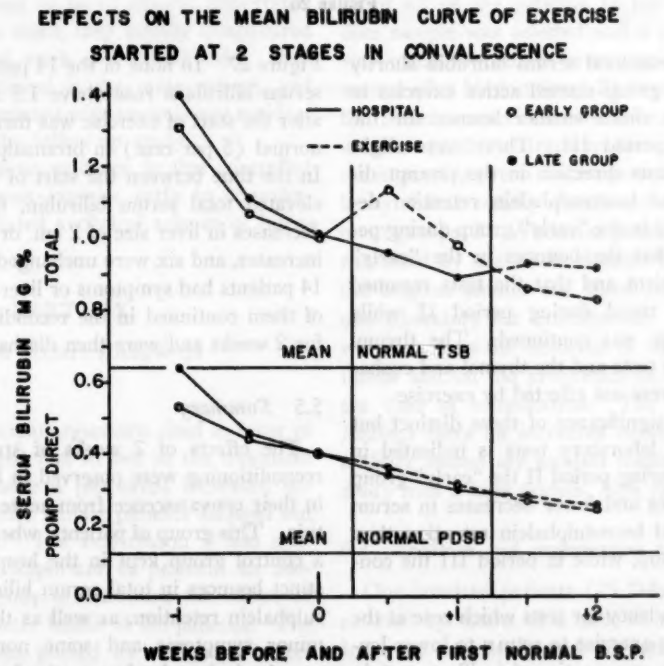

FIGURE 25

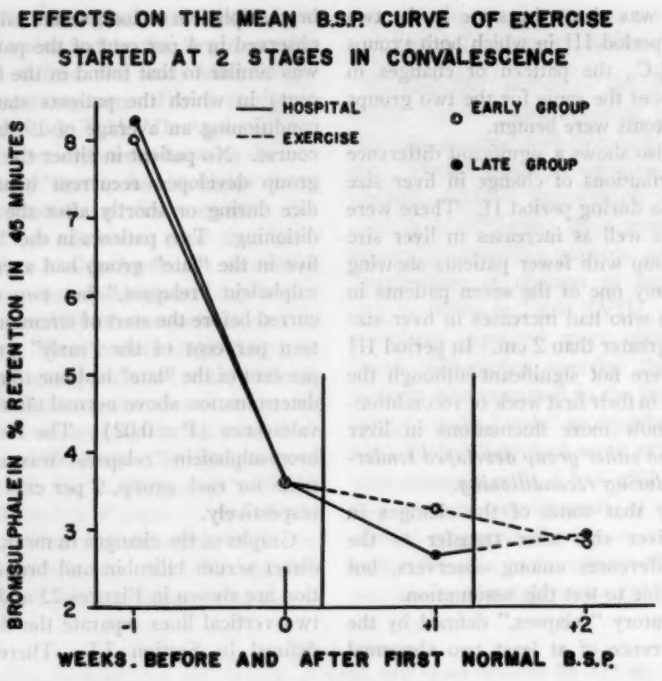

FIGURE 26

bounce in the mean total serum bilirubin shortly after the "early" group started active exercise in period II, and a much smaller bounce for the "late" group in period III. There were slight changes in the same direction in the prompt direct bilirubin, and bromsulphalein retention declined more slowly in the "early" group during period II. Note that the bounces in the "early" group were transient and that the tests resumed their downward trend during period II while strenuous exercise was continued. The thymol and zinc turbidity tests and the thymol and cephalin flocculations were not affected by exercise.

The statistical significance of these distinct but small changes in laboratory tests is indicated in Table XVIII. During period II the "early" group had more increases and fewer decreases in serum bilirubin level and bromsulphalein retention than did the "late" group, while in period III the converse was true.

The general tendency for tests which rose at the onset of strenuous exercise to return to lower levels while exercise was continued is illustrated in Figure 27. In none of the 14 patients whose total serum bilirubins rose above 1.5 mg. per 100 ml. after the start of exercise was there any rise above normal (5 per cent) in bromsulphalein retention. In the time between the start of exercise and the elevated total serum bilirubin, five patients had decreases in liver size of 2 cm. or more, three had increases, and six were unchanged. None of these 14 patients had symptoms or liver tenderness. All of them continued in the reconditioning program for 2 weeks and were then discharged to duty.

5.5 *Summary*

The effects of 2 weeks of strenuous physical reconditioning were observed in 88 patients early in their convalescence from acute infectious hepatitis. This group of patients, when compared with a control group kept in the hospital, showed distinct bounces in total serum bilirubin and bromsulphalein retention, as well as the appearance of minor symptoms and some non-tender hepatomegaly during the first week of reconditioning.

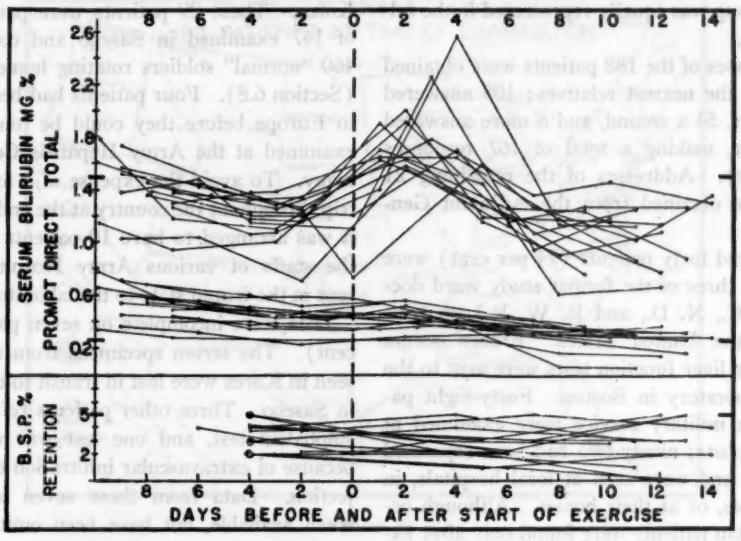

FIG. 27. CHANGES IN THE SERUM BILIRUBIN AND BROMSULPHALEIN TESTS OF THE 14 PATIENTS IN THE "EARLY" EXERCISE GROUP WHOSE TOTAL SERUM BILIRUBIN ROSE ABOVE NORMAL

Although statistically significant, these changes were not considered to be of clinical importance because they were small, they usually disappeared during the second week of reconditioning, they were seldom combined in the same patient, and they were not associated with unequivocal residual abnormalities in the follow-up study.

There was thus no evidence in this controlled study that strenuous exercise early in convalescence had precipitated a clinical relapse of infectious hepatitis.

PART SIX

FOURTH STUDY: FOLLOW-UP

6.1 *Design*

One of the principal arguments cited in favor of strict and prolonged rest in bed in the treatment of hepatitis has been a purported increased occurrence of disability among patients allowed out of bed "too soon." For this reason it was considered especially important to perform an adequate follow-up study to determine the effects of treatment on the later health of the patients. The following principles guided its design and execution: 1) Rather than attempt to obtain some information of varying completeness and accuracy about all of the patients in the studies, a random sample was selected and a vigorous attempt was made to contact every patient selected, no matter what his location. 2) It was assumed that the patients examined would be a representative sample and that extraneous factors and events in the interval between discharge and follow-up would be by chance equally distributed among the treatment groups. 3) Whenever possible patients were examined by one of the doctors who took part in the original studies and blood sera were sent to one laboratory for examination. 4) Conclusions were based on the incidence of significant interval illness and on the prevalence of abnormalities at the time of examination. *The only significant findings were an increased occurrence of abnormalities among the patients treated in the second study with a high protein, high carbohydrate diet.*

6.2 *Location and examination of patients*

One hundred patients (25 "blocks" of 4) were selected by means of a table of random numbers from the first study and 88 patients (11 "replicates" of 8) from the second study. Thus each

treatment group was equally represented in the follow-up study.

The addresses of the 188 patients were obtained by letters to the nearest relatives; 105 answered the first letter, 54 a second, and 8 more answered a third letter, making a total of 167 responses (89 per cent). Addresses of the remaining 21 patients were obtained from the Adjutant General's office.

One hundred forty patients (74 per cent) were examined by three of the former study ward doctors (J. G. C., N. D., and R. W. R.) on trips throughout the United States. Frozen serum specimens for liver function tests were sent to the follow-up laboratory in Boston. Forty-eight patients still in military service were examined at their Army posts; ninety-two had been separated from service and were seen at local hospitals, in doctors' offices, or at their homes. Although occasional civilian patients were found only after extensive inquiries at local post offices and corner drug stores, none refused to cooperate once located.

Twenty-nine patients (15 per cent) who would not have returned to the United States before the estimated closing date of the study were examined by one of the authors in Sasebo, Japan, or in Korea. These 29 patients were part of a group of 147 examined in Sasebo and compared with 460 "normal" soldiers rotating home from Korea (Section 6.8). Four patients had been reassigned to Europe before they could be found and were examined at the Army Hepatitis Center in Germany. To avoid the expense of a final circuitous trip throughout the country at the end of the study, it was arranged to have 10 patients examined by the staffs of various Army Hospitals and sera sent in the frozen state to the laboratory in Boston.

Data were incomplete on seven patients (4 per cent). The serum specimens from three patients seen in Korea were lost in transit to the laboratory in Sasebo. Three other patients refused a bromsulphalein test, and one test was unsatisfactory because of extravascular infiltration during the injection. Data from these seven are presented when available, but have been omitted from the final abnormality classifications.

A total of five patients (3 per cent) were not seen. One is somewhere in California; one is working as a merchant seaman; three were missed in the Far East and did not rotate home until after the study was completed.

TABLE XIX

Distribution of patients according to treatment group and location of follow-up examination

Treatment group†	Examined by research team: USA	Examined by research team: Japan or Korea	Examined by others	Not seen	Total selected for follow-up	Total seen
A. First study						
DR	20	3	1	1	25	24
Dr	21	1	3		25	25
dR	18 (1*)	2	4 (1*)	1	25	24
dr	21	1	2	1	25	24
Total	80	7	10	3	100	97
B. Second study						
Cps	11				11	11
CpS	6	4 (1*)		1	11	10
CPs	4	7 (2*)			11	11
CPS	7	3		1	11	10
cps	8	2	1		11	11
cpS	9 (1*)	1	1		11	11
cPs	7	3 (1*)	1		11	11
cPS	8	2	1		11	11
Total	60	22	4	2	88	86
Total for both studies	140 (2*)	29 (4*)	14 (1*)	5	188	183

* Numbers in parentheses refer to patients in whom data were incomplete.
† Capital letter indicates forced or high level treatment group, small letter the *ad lib.* or low level of treatment. D = diet; R = rest; C = carbohydrate; P = protein; S = supplements.

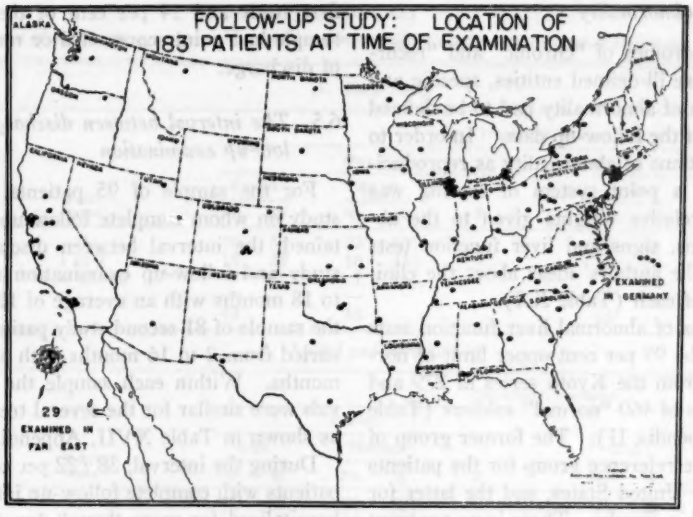

FIGURE 28

Outline map reprinted by permission of the American Map Company, Inc., New York.

Thus some information was obtained on 183 (97 per cent) of the 188 patients in the random sample, and complete information with reliable laboratory data on 176 (94 per cent). The distribution of the above patients by treatment groups is presented in Table XIX. The widely scattered geographic distribution of the patients at the time of examination is illustrated in Figure 28.

TABLE XX

Definition of the point system for classification of abnormalities found in the follow-up examinations

Finding	Severity	Point score
Symptoms: (anorexia, nausea, vomiting, malaise, liver pain)	No symptoms, or one mild symptom	0
	One moderate symptom and/or two or more mild symptoms	1
	Two or more symptoms of moderate or greater severity	2
Signs:		
Liver size	Not palpable or felt less than 3 cm. below right costal margin (corrected for location of upper border)	0
	Palpable 3 to 5 cm. below costal margin	1
	Palpable 6 cm. or more below costal margin	2
Liver tenderness	Tender to palpation or fist percussion	1
Spider angiomata	More than one	1
Spleen size	Any palpable enlargement	1
Laboratory tests:		
Bromsulphalein retention	More than 5% in 45 minutes	2
Total serum bilirubin	Above 1.08 mg./100 ml. (Far East)	
	Above 1.50 mg./100 ml. (U. S. A.)	2
Prompt direct serum bilirubin	Above 0.19 mg./100 ml. (Far East)	
	Above 0.26 mg./100 ml. (U. S. A.)	2
Thymol turbidity	Above 5.5 Shank-Hoagland units (Far East)	
	Above 6.5 Shank-Hoagland units (U. S. A.)	1
Cephalin flocculation	More than 2+ in 24 hours	1
Thymol flocculation	More than 2+ in 24 hours	1
Maximum points obtainable by one patient		16

6.3 *Criteria of abnormality*

Since the syndromes of "chronic" and "recurrent" hepatitis are ill-defined entities, specific and arbitrary criteria of abnormality had to be defined before analysis of the follow-up data. In order to make the evaluations of abnormality as reproducible as possible, a point system of scoring was evolved. The relative weights given to the abnormal symptoms, signs and liver function tests were based on the authors' ideas about the clinical importance of each (Table XX).

The definitions of abnormal liver function tests were based on the 99 per cent upper limit of normality derived from the Kyoto series of 279 and the Sasebo series of 460 "normal" soldiers (Table I, Text and Appendix II). The former group of normals were the reference group for the patients examined in the United States, and the latter for the 29 examined in Sasebo. There is no apparent explanation for the slight differences between the two series of normals.

6.4 *Effectiveness of the randomization*

The effectiveness of the method of random sampling used for this study was tested by comparing the follow-up sample with the remainder of the patients with respect to the mean values of three quantitative variables—age on admission to the hospital in Kyoto, total serum bilirubin on admission, and duration of acute illness from admission to convalescence (Table XVII, Appendix II). Unfortunately, there were significant departures from randomness in the samples drawn from each of the original studies. For each discrepancy, however, the sample was either older than the remainder, or the patients were more severely ill during the acute course, or both. Hence, any bias introduced by the sampling procedure might be expected to increase the likelihood of residual disability in the sample, rather than to lead to underestimation of the occurrence of abnormalities. Within the sample the treatment groups were reasonably comparable with respect to point scores at the time of discharge to duty. The average scores of 1 and 2 points (see Table XVII, Appendix II) reflect the fact that the majority of patients in these studies were allowed to return to duty with a variety of minor abnormalities. In fact, 16 per cent of the follow-up sample from the first study and 14 per cent of the second study sample had point scores of 3 or more at the time of discharge.

6.5 *The interval between discharge and the follow-up examination*

For the sample of 95 patients from the first study on whom complete follow-up data were obtained, the interval between discharge from the study and follow-up examination ranged from 7 to 18 months with an average of 12 months. For the sample of 81 second study patients the interval varied from 2 to 16 months with an average of 8 months. Within each sample the average intervals were similar for the several treatment groups, as shown in Table XVII, Appendix II.

During the interval, 38 (22 per cent) of the 176 patients with complete follow-up information were hospitalized for more than 1 day (Table XVIII, Appendix II). In only 19 of these patients was the loss of time from duty or work associated with gastrointestinal or abdominal complaints and in only nine did it seem at all likely that the "disability" might have been related to the previous hepatitis. Attempts were made to obtain the medical and hospital records for these nine instances of interval illness, and eight records or abstracts were received. In four of the eight patients no evidence of residual or recurrent hepatitis had been found during hospitalization. The remaining four patients had been found to have physical and/or laboratory abnormalities which suggested recurrent or chronic hepatitis, but in no instance was there definite evidence for a diagnosis of "infectious hepatitis with jaundice." Interval disability attributable to the patients' hepatitis was so rare, therefore, that its occurrence could not be used to compare the treatment regimens.

6.6 *Comparisons of treatment groups at the time of follow-up examinations*

A) *Specific abnormalities.* The individual abnormalities listed in Table XIX, Appendix II, occur more commonly among the *ad lib.* rest group in the first study but this difference is less striking when the abnormalities are graded according to clinical importance in Table XXI below. The dietary treatment groups in the first study are similar.

TABLE XXI

Comparison of treatment regimens with respect to abnormalities existing at the time of follow-up examinations *

A. First study

Status on follow-up	Point score	Treatment regimen: Diet — Forced No.	Forced %	*Ad lib.* No.	*Ad lib.* %	P	Rest — Strict No.	Strict %	*Ad lib.* No.	*Ad lib.* %	P
Normal	0	26	53	23	50		28	61	21	43	
Equivocal	1 or 2	16	33	16	35	.9	12	26	20	41	.2
Abnormal	3 or above	7	14	7	15		6	13	8	16	
Total patients		49		46			46		49		

B. Second and third studies

Status on follow-up	Point score	Therapeutic regimens: Protein — High No.	High %	"Normal" No.	"Normal" %	P	Calories — High No.	High %	"Normal" No.	"Normal" %	P
Normal	0	17	42	28	68		24	62	21	50	
Equivocal	1 or 2	18	45	12	29	.04	10	26	20	48	.05
Abnormal	3 or above	5	13	1	3		5	12	1	2	
Total patients		40		41			39		42		

Status on follow-up	Point score	Supplements — Added No.	Added %	Placebos No.	Placebos %	P	Exercise — "Early" No.	"Early" %	"Late" No.	"Late" %	P
Normal	0	19	48	26	63		22	55	23	56	
Equivocal	1 or 2	17	42	13	32	.3	14	35	16	39	.7
Abnormal	3 or above	4	2	2	5		4	10	2	5	
Total patients		40		41			40		41		

C. Comparison of first and second studies

Status on follow-up	Point score	First study No.	First study %	Second study No.	Second study %	P
Normal	0	49	52	45	56	
Equivocal	1 or 2	32	34	30	37	.3
Abnormal	3 or above	14	15	6	7	
Total patients		95		81		

D. Ward comparison—second study

Status on follow-up	Point score	High pro. High cal.	High pro. "Normal" cal.	"Normal" pro. High cal.	"Normal" pro. "Normal" cal.
Normal	0	6	11	18	10
Equivocal	1 or 2	9	9	1	11
Abnormal	3 or above	4	1	1	0
Total patients		19	21	20	21

* Data complete on 176 of the 188 patients selected for follow-up.

In the sample of patients from the second and third studies there was a tendency for those in the high-protein, high-calorie treatment group (CP) to present more abnormal findings for most of the measurements, but the individual differences were not large. The patients who had received vitamin and choline supplements were similar to those who had been given placebos. Among patients from

the "early" reconditioning group there were eight instances of liver tenderness while the "late" group had none, but these two groups were similar with respect to all of the other variables. The second study patients, all treated with *ad lib.* rest, had a slightly lower prevalence of abnormalities than the patients of the first study.

B) *Abnormalities by point scores.* Arbitrarily, those patients with no abnormality points were designated "normal," those with 1 or 2 points "equivocal," and those with 3 or more points "abnormal." Comparisons among treatment groups have been made for the percentage distributions of patients in these three categories and "chi-square" tests of significance have been applied with the results shown in Table XXI. There were no significant differences among the treatment groups in the first study. In the second study the high-protein regimen produced significantly more equivocal and abnormal patients than the "normal"-protein diets, while the high-calorie diets tended to yield more abnormals than the "normal"-calorie groups. The table of ward comparisons demonstrates that these differences were largely due to the poor showing of a single ward (CP) on which the high-protein, high-calorie diets were served. The third study comparison of "early" and "late" reconditioning groups did not demonstrate any striking differences. A comparison of the first and second studies showed no significant differences.

6.7 *Results of additional follow-up examinations*

Eleven patients were hospitalized in the United States for further investigation of abnormalities found in the follow-up examination in either the Far East or the United States. Ten of them were sent to Walter Reed Army Hospital and one to the Veterans Administration Hospital in Phoenix, Arizona.

All but one of the patients had improved by the time they were admitted for study, the interval between the routine follow-up examination and

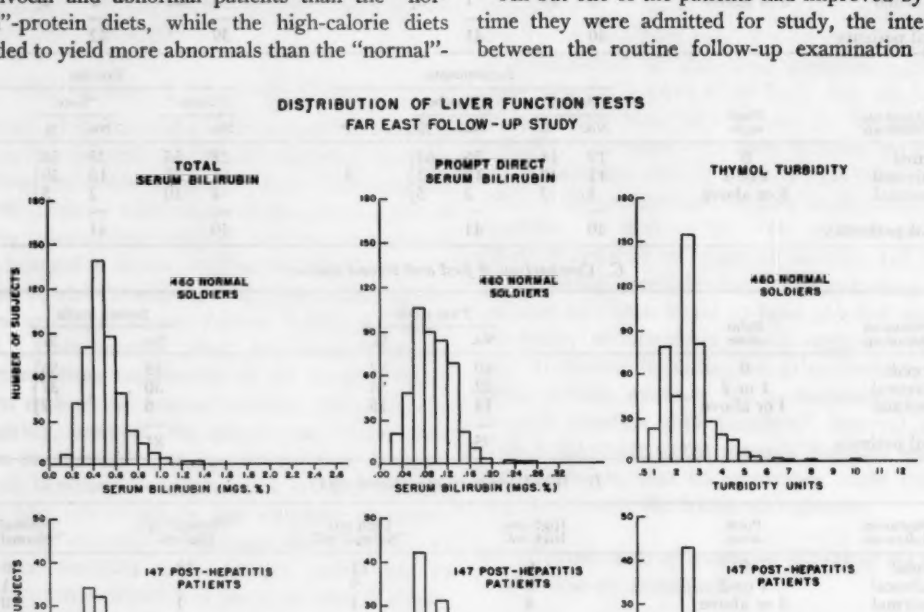

FIG. 29. COMPARISON OF LIVER FUNCTION TESTS IN 147 FORMER HEPATITIS PATIENTS ROTATING HOME FROM KOREA THROUGH SASEBO, JAPAN, AND 460 "NORMAL" SOLDIERS

TABLE XXII

Physical findings of 147 post-hepatitis patients compared with 460 "normal" soldiers

	Post-hepatitis		"Normal" controls		
	Number	%	Number	%	P
Liver not palpable	102	69	405	88	
1–2 cm.	20	14	27	6	
3–5 cm.	22 } 25	15 } 17	25 } 28	5 } 6	<.001
>5 cm.	3 }	2 }	3 }	1 }	
Liver tenderness	15	10	2	0.5	.01
Spleen palpable	2	1	5	1	—
Spider angiomata	13	8	10	2.5	.02

subsequent hospitalization varying from 1 to 3 months. Eight of the 11 patients had liver biopsies; the livers of seven were essentially normal and changes consistent with "chronic active hepatitis" were noted in one. Discharge diagnoses were "no liver disease" in eight of the patients, "inactive hepatitis" in two, and "chronic hepatitis" in one.

Since many of the factors which led to the hospitalization of these particular patients were beyond the control of the research team, these results are not intended for use in the interpretation of the effects of previous therapy on the occurrence of residual abnormalities. However, these supplementary hospital studies certainly suggest that the arbitrarily defined "abnormalities" found in the follow-up study may have little clinical significance.

6.8 *Comparisons of post-hepatitis patients with "normal" soldiers*

Because of the possibility that sufficient of the randomly selected patients could not be found in the follow-up study outlined above, facilities were set up to examine former patients as they rotated through Sasebo, Japan, on their way back to the United States. Since the selection of patients examined did not depend on chance alone, the results could not be used to assess the efficacy of the various treatments. The former patients, however, could be compared with rotating soldiers who had not had hepatitis during their stay in the Far East.

TABLE XXIII

Distributions of urine urobilinogen in 115 post-hepatitis patients and 50 "normal" soldiers

Ehrlich units *per hour*	Post-hepatitis		"Normals"	
	Number	%	Number	%
0.10–0.29	14	12	4	8
0.30–0.49	39	34	11	22
0.50–0.69	33	29	20	40
0.70–0.89	11	10	7	14
0.90–1.09	8	7	6	12
1.10–1.29	2	2	0	0
1.30–1.49	2	2	2	4
1.50–1.69	3	3	0	0
1.70–1.89	2	2	0	0
>1.89	1	1	0	0
Total patients	115		50	

One hundred and forty-seven of the former patients and 460 "normals" were examined in Sasebo by one of the authors (C. W. S.). Liver function tests were carried out by one of the former Hepatitis Center technicians.[21] The 2-hour uro-

[21] Thanks are due Dr. Noriaki Wakizaka for performing the laboratory determinations.

TABLE XXIV

Distributions of cephalin and thymol flocculation tests in 147 post-hepatitis patients and 460 "normal" soldiers

	Cephalin flocculation					Thymol flocculation				
	Post-hepatitis		"Normals"			Post-hepatitis		"Normals"		
	Number	%	Number	%		Number	%	Number	%	
0	134	91	413	90	0	119	81	410	89	
1+	10	7	43	9	1+	22	15	34	7	
2+	2	2	3	1	2+	4	3	14	3	
3+	1	1	0	0	3+	2	1	2	—	
4+	0	0	1	—	4+	0	0	0	0	
Total	147		460			147		460		

TABLE XXV

Comparisons of the means of liver function tests in the 147 post-hepatitis patients and 460 "normal" soldiers

Test	Post-hepatitis patients	"Normal" soldiers
Serum bilirubin—*mg./100 ml.*		
Total	0.51	0.50
Prompt direct	0.09	0.09
Thymol turbidity		
Shank-Hoagland units	3.0	2.5
Urine urobilinogen*		
Ehrlich units per hour	0.62	0.63

* This test was done on only 115 post-hepatitis patients and 50 "normal" soldiers.

bilinogen test (30) was added to the tests previously performed. The patients were examined from 2 to 15 months after discharge from the hospital (mean 8 months). Eighty per cent had been discharged more than 6 months previously.

Thirty-one of the 147 patients (21 per cent) complained of various combinations of anorexia, nausea, vomiting, malaise, fatigue, and pain. Symptoms at the time of the examination of the "normal" patients were not recorded, but it seems likely that they had fewer complaints than the hepatitis patients. Physical signs (Table XXII) were more common in the post-hepatitis group, the most striking difference being the frequency of hepatic tenderness. Liver function tests in the two groups are compared in Figure 29 and in Tables XXIII, XXIV and XXV. The distributions and means of the total and prompt direct serum bilirubin, cephalin flocculation and thymol flocculation, thymol turbidity and urine urobilinogen were quite similar for the post-hepatitis and "normal" groups.

6.9 *Summary*

1. One hundred and eighty-eight patients were selected at random from the original group of 450 for follow-up examinations. Ninety-seven per cent, or 183 of the patients, were examined and complete information was obtained on 94 per cent (176 patients).

2. Approximately 5 per cent of the sample, equally distributed among the treatment groups, lost more than one day from duty or work because of complaints which were possibly attributable to hepatitis, but no case of recurrent jaundice was seen.

3. The patients on the *ad lib.* rest regimen had more individual abnormalities than those on strict bed rest, but when these were graded according to clinical importance, the difference was not significant.

4. There was no difference between the patients treated with the forced high-protein and *ad lib.* diets in the first study. Those patients in the second study from the high-protein, high-calorie ward showed more equivocal and distinct abnormalities than the patients from the other three wards.

5. Subsequent hospitalization of 11 "abnormal" patients showed all but one to be essentially normal.

6. One hundred and forty-seven former patients were compared with 460 "normal" controls as they rotated through Sasebo, Japan, on their way back to the United States. The patients showed an increased frequency of symptoms, hepatomegaly, liver tenderness and spider angiomata, but the distributions of liver function tests were the same as in the controls.

PART SEVEN

RETROSPECTIVE ANALYSES OF THE EFFECTS OF REST AND DIET

The results presented in Parts Three and Four are those of controlled experiments in which the patients were assigned to treatment groups at random and the general plan of analysis was decided before the results were available. During the conduct of these controlled studies, numerous subsidiary data were gathered in the expectation that their analyses might further elucidate any treatment effects demonstrated. This section is concerned with retrospective studies suggested by the data or by the conclusions of others. The patients have been grouped for analysis not at random before the start, but systematically and "after the fact." Therefore, the results should be looked upon as suggestive rather than conclusive, and of limited value from the standpoint of probability statements. ***The results are generally negative and in no way qualify the conclusions drawn from the first and second studies.***

TABLE XXVI

First study: Relation of rest effects to the duration of symptoms before admission and the height of the total serum bilirubin on admission

	Strict bed rest			*Ad lib.* rest		
	Number of patients	Mean adm. TSB	Mean duration of illness	Number of patients	Mean adm. TSB	Mean duration of illness
Symptoms 10 days or less	71	7.7	30	73	7.9	26
Symptoms 11 to 21 days	55	7.7	32	54	8.6	28
Admission TSB 1 to 6.9 mg./100 ml.	70	4.1	23	58	3.8	20
Admission TSB 7 to 12.9 mg./100 ml.	39	10.0	37	48	9.6	31
Admission TSB 13+ mg./100 ml.	17	17.8	42	21	17.8	45

7.1 *Relation of the stage and severity of illness to the effects of rest*

The belief that strict bed rest should be continued after the patient with acute hepatitis no longer desires to stay in bed is so firmly entrenched in current medical practice that the failure to confirm it in the first study warrants some further inspection of the data. To investigate whether enforced bed rest might still be essential early in the course of the disease the patients were divided into two approximately equal groups: those with any symptoms for 10 days or less before admission and those who had been sick for from 11 to 21 days. The data presented in Table XXVI reveal that the *ad lib.* rest patients had a shorter duration of illness in the hospital irregardless of how long they had been sick before admission. When the patients were divided according to the severity of illness as represented by the total serum bilirubin on admission (Table XXVI) the *ad lib.* rest groups had shorter durations in the low and medium bilirubin ranges, but slightly longer durations in the high ranges. None of these differences is significant.

7.2 *The effects of pre-hospitalization activity on the duration of subsequent illness*

An attempt was made to record on admission to the hospital each patient's activity on each day of his illness before admission. The number of days in which the patient's activity was more than one-third his usual activity, from the onset of symptoms and from the onset of jaundice to hospitalization, were then plotted in scattergrams against the duration of the acute illness in the hospital. No suggestive correlations were apparent.

7.3 *Relation of the stage and severity of the illness to the effects of diet*

An early dietary effect was demonstrated in both of the present studies by the fact that the trends of the mean serum bilirubins began to diverge within 3 to 4 days of the start of treatment (Figures 10 and 26). This early effect was further illustrated by the fact that the *ad lib.* and "normal"-protein groups each had significantly more patients whose serum bilirubins rose after admission to the hospital. Thus, in the two studies there were 35 patients on the *ad lib.* and "normal"-protein regimens with rising bilirubin levels and only eight on the forced and high-protein diets (see Table VIII, Appendix II). In 27 (63 per cent) of these 43 patients, the total serum bilirubin had risen 1 mg. per 100 ml. or more by 4 days after admission, and in 37 (86 per cent) the rise was apparent by the end of the first week. If one therefore assumes that the difference in percentage of patients with rising serum bilirubins is a distinct treatment effect rather than chance occurrence, and this seems likely in view of the fact that a difference of equal magnitude and direction was found in both studies, then one must also assume that the dietary effect takes place, at least in part, in the first week of treatment, or during the acute stage in which the serum bilirubin may still be rising.

To investigate further the relationship between the observed dietary effects and the stage of illness in which treatment was started, the patients in

TABLE XXVII

Relation of dietary treatment effects to duration of symptoms before admission

	First study			Second study		
	Forced diet	*Ad lib.* diet	Per cent shortening of illness by forced diet	High protein diet	"Normal" protein diet	Per cent shortening of illness by high protein diet
Symptoms 10 days or less:						
Number of patients	69	75		47	51	
Mean admission TSB	7.2	8.4		8.5	8.1	
Mean duration of illness	24	31	23	28	35	20
Symptoms 11 to 21 days:						
Number of patients	59	50		47	44	
Mean admission TSB	9.0	7.1		8.8	8.2	
Mean duration of illness	28	34	18	30	34	12

each study were divided into two groups, those who had symptoms for 10 days or less before admission and those who had been sick from 11 to 21 days. The data are presented in Table XXVII. The overall duration of illness does not differ in those sick a short or a long time before admission. In both studies, however, the dietary effect is slightly greater in those admitted to the study early in the course of their illness.

To determine whether the treatment effect might vary according to the height of the admission total serum bilirubin, the patients were divided into three groups, those with low, moderate, and high admission levels (Table XXVIII). In both studies the treatment effect was greatest in the middle range of bilirubin levels. It was least apparent at the high bilirubin levels, but the number of patients in this group was small.

For practical purposes the severity of the illness might also be judged by the magnitude of the patient's complaints and the degree of hepatomegaly on admission. The results of analyses of dietary treatment effects in the second study with respect to these criteria of severity are presented in Table XXIX. Since both measurements are susceptible to "doctor effects" (see Sections 3.1 and 5.1), the patients on each ward were ranked according to severity of symptoms and liver size and then divided at the middle rank into two groups designated as "severe" and "mild." The patients with the more severe symptoms and larger livers did have higher admission bilirubin

TABLE XXVIII

Relation of dietary treatment effects to the total serum bilirubin on admission

	First study			Second study		
	Forced diet	*Ad lib.* diet	Per cent shortening of illness by forced diet	High protein diet	"Normal" protein diet	Per cent shortening of illness by high protein diet
Admission TSB (1 to 6.9 mg./100 ml.)						
Number of patients	64	64		44	50	
Mean admission TSB	3.8	4.1		—	—	
Mean duration of illness	19	25	24	24	28	14
Admission TSB (7 to 12.9 mg./100 ml.)						
Number of patients	42	45		32	27	
Mean admission TSB	9.4	10.0		—	—	
Mean duration of illness	28	39	28	29	40	28
Admission TSB (13+mg./100 ml.)						
Number of patients	22	16		18	18	
Mean admission TSB	18.4	17.0		—	—	
Mean duration of illness	43	44	2	43	49	12

TABLE XXIX

Second study: Relation of dietary treatment effects to the severity of symptoms and size of the liver on admission

	High protein diet			"Normal" protein diet			Per cent shortening of illness by high protein diet
	Number of patients	Mean adm. TSB	Mean duration of illness	Number of patients	Mean adm. TSB	Mean duration of illness	
"Severe" symptoms*	46	9.4	31	47	8.9	38	18
"Mild" symptoms	48	8.0	27	48	7.5	30	10
"Severe" hepatomegaly*	48	9.3	31	48	8.5	36	14
"Mild" hepatomegaly	46	8.0	28	47	7.9	32	12

* To avoid the influence of "doctor effects," the patients on each ward were ranked according to symptoms and liver size and then divided at the middle ranks.

levels and slightly longer acute illnesses. The protein effect was the same regardless of the severity of the illness.

From the standpoint of severity of illness on admission the six patients on the forced-fed wards who required tube feedings were entirely similar to the nine patients on the *ad lib.* wards whose intake in the first 4 hospital days was less than 2,000 calories or 70 grams of protein. Yet the mean durations of illness of each group were respectively 33 and 51 days. Although the numbers involved are small, the figures suggest that the severely anorectic patients on the forced-diet wards might have had more prolonged illnesses if they had not been tube fed.

It should be pointed out, however, that none of the patients in these studies were in the precoma or comatose stages in which the *deleterious* effects of a high-protein diet on the central nervous system—in cirrhosis of the liver—have been demonstrated (31).

PART EIGHT

GENERAL SUMMARY, DISCUSSION, AND RECOMMENDATIONS

8.1 *Rest effects*

A. *Summary of results*

1. Patients hospitalized with acute infectious hepatitis improved as rapidly when allowed to be up and about their wards at will as did patients kept at strictly enforced bed rest.

2. Asymptomatic laboratory relapses occurred early in convalescence with equal frequency for both groups of patients.

3. There was no evidence that *ad lib.* ambulation was detrimental irrespective of the stage or severity of the patients' illness.

4. Patients started on an active physical reconditioning program early in convalescence had increases in symptoms, signs and laboratory tests of small degree and transient nature. Since they disappeared as exercise continued, they were not considered of clinical importance.

5. The incidence of chronic disability and significant residual abnormalities observed in a follow-up study was no greater for patients permitted *ad lib.* ambulation and started on strenuous physical rehabilitation early in convalescence than it was for patients treated in the more conventional conservative manner.

B. *Discussion of results*

The conclusion drawn from these studies that it is entirely unnecessary to enforce bed rest in the treatment of acute infectious hepatitis is at variance with the great majority of interpretations of previous data expressed in the literature (1, 2, 5, 28, 32). It is important, therefore, to discuss possible explanations for this discrepancy.

It does not seem plausible that the results of the present study are statistical artefacts. The study was well controlled, except for the fact that "ward and doctor effects" could not be completely excluded. There was no evidence that the strict bed rest patients were by chance more severely ill. The doctor who decided on the eligibility of a patient for admission to the study had no knowledge of the treatment he would receive until after he was admitted. Numerous randomization checks revealed the groups to be similar with respect to many variables other than the treatments.

Although as always in infectious hepatitis the variability in duration of the acute disease was large, so was the number of patients included in this study, and the "confidence limits" make it extremely unlikely that a clinically important difference in favor of strict bed rest would be observed on repetitions of the study under similar circumstances. Careful follow-up studies of the patients in the hospital, at the physical reconditioning center, in Japan at the time of their rotation to the United States, and after their return have revealed no evidence that the patients treated with *ad lib.* rest have had an increased incidence of significant residual abnormalities. Long-term effects must remain for the time being a matter for conjecture, but there is no suggestion in follow-up data now available on World War II patients (33) that long-term residuals will be common enough to reverse the present conclusion that strict bed rest is no longer necessary.

The lack of effectiveness of strict bed rest noted in this study did not appear to be due to significantly different clinical material or a milder form of the disease than reported in other epidemics. To illustrate this fact data from descriptions of epidemics of infectious hepatitis among military personnel in post-war Germany, and during World War II in the United States, Great Britain, Italy, and the Middle East (1–3, 34–36) are presented in Table XX, Appendix II. As might be expected, there was considerable variation in the manner in which the data were handled in each of these reports, and some liberties have been taken in the tabulation to make them as comparable as possible. Symptoms were essentially the same in all with the exception that in post-war Germany the onset has been appreciably more insidious. Within the limits of the different standards of measurement and methods of handling the patients, the duration of jaundice and hospitalization were similar in all of these epidemics. Certainly by these criteria the patients in the present studies were no less sick. To obtain quantitative data comparable to that of the present study, the laboratory records of 100 randomly selected patients[22] who had hepatitis in post-war Germany were reviewed. The admission total serum bilirubins and the duration from admission to a bilirubin of 1.5 mg. per 100 ml. or less are compared in Table XXX with 253 first study patients. The differences in the mean initial serum bilirubins and the durations of jaundice are not striking. It seems unlikely, therefore, that any such small differences between epidemics in comparable populations could result in a need for enforced bed rest in one group and not in the other.

The only striking difference between the present series of patients and those studied during World War II lies in the incidence of relapses. Return of jaundice within 2 or 3 months after it had once disappeared was never seen in the present study and occurred in 5 per cent or more of the patients studied in Great Britain (34) and Italy (1). This plus the fact that the precipitation of relapses by exercise early in convalescence was not observed in the present studies, as contrasted with the series in Italy (1), suggests that the epidemic behavior of infectious hepatitis may have changed since World War II. Another possible explanation for the relatively high incidence of relapses with jaundice during World War II may be that anorectic patients were occasionally treated with intravenous plasma which may have contained the virus of homologous serum jaundice. In addition, the transmission of the hepatitis viruses by

[22] The authors wish to thank Dr. W. P. Havens, Jr. for supplying these records.

TABLE XXX

Comparison of hepatitis patients in post-war Germany with first study patients

	Number of patients	Mean duration of illness before admission *days*	Mean admission TSB *mg. per 100 ml.*	Duration to first normal TSB*	
				Mean *days*	S.D. *days*
Japan and Korea (first study)	253	10	8.1	23	15
Germany	100	14	7.4	27	16

* 1.5 mg. per 100 ml. or less.

syringes and needles had not then been clearly demonstrated. Thus some of the relapses may have been reinfections with a different strain of virus.

Two other possibly important differences may be conjectured. First, more of the World War II patients may have had homologous serum jaundice and it is conceivable, although improbable, that in this disease bed rest is more effective than it is in infectious hepatitis. Finally, the patients in some World War II epidemics were probably more fatigued and less well fed when they acquired their hepatitis than the patients in the present studies. However, McFarlan (37) could demonstrate no relation between fatigue and diet before onset and the severity of the disease among British troops in World War II, and retrospective analyses of the present data revealed none.

If the conclusions of the present study are accepted, and the patient material and disease are not too different from that reported by others, a re-examination of the data on which the concept of strict bed rest has been based is indicated. Only two "controlled" studies are available for comparison (1, 5, 7), and in both of these studies the patients on bed rest who had a shorter duration of acute illness also received a high-protein diet, whereas patients on a modified rest program received an *ad lib.* diet. Thus the entire effect may have been due to diet.

In a retrospective study (2) interpreted as emphasizing the importance of bed rest, the total duration of illness for patients entering the hospital less than 30 days after onset was distinctly shorter than for those who were hospitalized after 30 days or more of illness. The patients were Navy personnel who were transferred to one hospital as soon as a diagnosis of hepatitis was made, and delays in transfer were attributed to delays in reaching port or difficulties in establishing the diagnosis. Since the pre-admission regimen was assumed to be one of *ad lib.* rest and all patients were kept strictly in bed after admission, it was concluded that restriction of activity early in the illness had appreciably shortened the duration. An equally plausible explanation for the difference has nothing to do with restriction of activity. It seems probable that the original group of patients, who could not be transferred early for various reasons, included some who recovered within 30 days and therefore never were transferred. Thus the group of late transfers may well have been overloaded with patients who were destined to have prolonged illnesses irrespective of how they were treated.

Another type of retrospective observation on which a great deal of emphasis has been placed is exemplified by a case report (1) of a patient whose icteric index was approximately 25 on admission, fell to normal during 3 weeks of bed rest, and rose to 70 after 2 weeks of exercise about the ward. Such time-related events may lead to useful hypotheses to be tested in controlled studies, but by themselves are not sufficient evidence for a cause and effect relationship.

Recently two "controlled" experiments from the Army Hepatitis Center in Germany have suggested that, at least in the type of patient material encountered there, rest did not have to be as prolonged as heretofore thought necessary. In the first (3), patients were exercised mildly and cautiously at all stages in the disease and adverse effects were encountered only in those with total serum bilirubins above 3 mg. per 100 ml. In the second (4), the effects of physical reconditioning in patients with persistent bromsulphalein retentions between 5 per cent and 10 per cent were compared with the effects in patients with less than 5 per cent retention, and no differences were found. It was suggested that mild elevation of the bromsulphalein test in the presence of a total serum bilirubin of 1.0 mg. per 100 ml. or under should not be a contraindication to the start of ambulation. No one, however, has previously suggested that bed rest should be *ad lib.* throughout the illness.

It should be emphasized that the ambulatory patients in the present studies were never forced to stay out of bed or to perform any labor, and were confined to their hospital wards. *The study offers no evidence, therefore, that rest is not advantageous when the patient feels sick.* It is still probable that enforced activity during the acute phase of hepatitis, as in any infectious disease, will make the patient feel worse, and may possibly prolong the disease. It should also be emphasized that the regimen of *ad lib.* rest was not instituted until an average of 10 days after the onset of symptoms, 7 days after the onset of dark urine and 4 days after first hospitalization. The data in Table

XXVI, however, suggest that the time of starting treatment is not an important factor.

The third study satisfactorily demonstrated that patients who have been at *ad lib.* rest throughout their acute illness may be returned with safety to their normal activity as soon as their total bilirubins have been 1.5 mg. per 100 ml. or less for 1 week and their bromsulphalein retention 5 per cent or less. Exercise started at this time did not result in an increased incidence of relapses or residual disability. That this regimen of *ad lib.* rest in bed and earlier return to duty results in a substantial saving in the duration of hospitalization is illustrated by the following data: The average duration of hospitalization of all patients transferred to the physical reconditioning center in the first study was *56 days;* in the second study the 94 patients in the early reconditioning group were discharged after *34 days.* Thus, disregarding any potential dietary savings, 22 days were eliminated from the duration of hospitalization. Part of this saving in time may be attributed to the elimination of all possible administrative delays and the postponement of elective minor surgery and dental work until reconditioning had begun. The greatest saving, however, resulted from the elimination of the physical disability that results from prolonged rest in bed.

8.2 *Diet effects*

A. *Summary of results*

1. In the first study, patients forced to eat a diet supplying an abundance of calories, protein and vitamins throughout hospitalization improved more rapidly than did those patients who ate only as much as they chose of a regular hospital diet.

2. In the second study it was observed that the duration of hepatitis was shorter for those patients forced to eat a high-protein diet (19 per cent protein calories) than for those who consumed the "normal"-protein diet (11 per cent protein calories). The duration of illness was similar for patients eating 3000 and 4000 calories, and for those who received supplemental choline and vitamins and those who did not.

3. Asymptomatic laboratory relapses occurred with equal frequency irrespective of the composition of the diets.

4. During the first 2 weeks of treatment, there was a more rapid decline of the serum bilirubin in the forced-diet groups of the first study, and in the high-protein groups of the second study.

5. Although first study patients (forced and *ad lib.*) were similar when seen in follow-up, second study patients who received the high-protein and high-calorie diet showed a slightly higher incidence of mild residual abnormalities.

B. *Discussion of results*

The 22 per cent reduction in the duration of the acute disease by the forced diet noted in the first study was highly significant. This indicates that repetitions of the study would almost certainly yield similar dietary results. From the clinical standpoint, however, this reduction is not very impressive when compared with the effects of more specific therapy in other infectious diseases. In civilian medicine the potential saving of a week on the average, in the absence of any evidence for the prevention of death or chronicity, might not be worth the extra trouble of forcing the patient to eat. From the military standpoint, however, an average saving of 1 week during an epidemic period when thousands of cases occur each year represents a substantial saving in man power and expense.

The second study was designed, in part, to gain some information as to whether the absolute amount of protein or percentage of protein calories was more important. The variability in duration of illness encountered in the second study was so large in relation to the number of patients available that this problem could not be specifically solved. But a reorientation of results from the second study supplied some evidence for discussion. Table XXXI shows that there was a linear decrease in duration of the disease as the amount of protein in the diet was increased. Although this comparison was not included in the original design of the study and disregards caloric levels, it does suggest that actual content of protein was important in shortening the duration of illness. On the other hand, as shown in Table XI, the protein effect was the same at both caloric levels, indicating the effect of percentage composition of the diet. The question cannot therefore be answered by the data of this study.

The smaller dietary effects encountered in the

TABLE XXXI

Second study: Relation of protein content of diet to duration of illness

		Number of patients	Protein content		Calories	Mean duration of illness
			grams	% of calories		
High calorie, high protein	(CP)	42	190	19	4,000	28
"Normal" calorie, high protein	(cP)	42	140	19	3,000	30
High calorie, "normal" protein	(Cp)	42	110	11	4,000	33
"Normal" calorie, "normal" protein	(cp)	42	80	11	3,000	35

second study than in the first, and the differences in follow-up findings, make desirable an examination of other differences between the two studies. Data presented in Table V, Appendix II, suggest that the patient material differed in the two studies. The mean duration of symptoms and of jaundice before admission were slightly longer in the second study, although the duration of hospitalization elsewhere was the same. In spite of the fact that they were admitted somewhat later in their course, significantly more of the patients in the second study complained on admission of malaise, nausea, and vomiting. The mean prompt direct serum bilirubin on admission was significantly higher in the second study. Conversely, the mean thymol and zinc turbidities were lower, as were the percentages of patients with positive cephalin and thymol flocculations. During the period of the second study fewer patients became ill while in active combat, there were fewer instances suggestive of "point epidemics" in combat units than in the first study, and the admission rate at the Hepatitis Center was lower. Perhaps related to these changes the variability of durations of illness was somewhat greater in the second study than in the first. However, the duration of illness in the hospital was about the same in both studies, the overall geometric means being 25 days in the first study and 27 days in the second.

Since dietary intakes of patients in the second study were restricted to narrow ranges, the high-level diets differed from the forced diet in the first study in that the minimum levels were higher and there were no patients eating more than the prescribed amounts. The "normal" diets were unlike the *ad lib.* diet in the first study in that there were no patients eating less than 2700 calories or 70 grams of protein, and there were no excessive intakes. Thus the dietary intakes were different enough to make a comparison of the effects in the two studies unwarranted.

Whatever the reasons for these differences between the two studies and whatever their influence on the treatment effects, the fact remains that the effect of the high-protein diet on the duration of illness in the second study was not very much better than that of the "normal"-protein diet, and even that advantage must be qualified by the results of the follow-up study.

The beneficial results arising from the forced feeding of a diet rich in protein which were demonstrated so conclusively in these studies are difficult to reconcile with studies recently reported by Leone and his co-workers (38). These workers have obtained some evidence from observations on prisoner volunteers participating in studies of viral hepatitis that a high-protein, low-fat diet started within a few days of the onset of symptoms may increase the severity and prolong the duration of illness. There are several obvious differences between the dietary study on prisoners and the present studies, one or more of which might contribute to the divergent results. It seems unlikely, in view of the data presented in Tables XXVIII, XXIX, and XXX, that the differences in the conclusions of the two studies can be explained by the factors of time of starting treatment and the severity of illness. The possibility of differences due to the type of virus or to the fat content [23] of the special diets of Leone may warrant further investigation.

[23] In a series too small for statistical validity, Hoagland, Labby, Kunkel, and Shank (6) found that 15 patients on a high-protein, low-fat diet had an acute illness averaging 8 days longer than an equivalent number on a high-protein, high-fat diet.

8.3 *Recommendations*

When the results of the above studies and those of Leone, and his co-workers (38) were presented to the Committee on Hepatitis of the Commission on Liver Disease of the Armed Forces Epidemiological Board, recommendations were drawn up for revision of the treatment of acute infectious hepatitis in the Armed Forces. The following recommendations were therefore sent by the Epidemiological Board to The Surgeon Generals of the Armed Forces:

The following recommendations for the treatment of acute infectious hepatitis are general principles and may require modification for individual patients under particular circumstances. They embody a distinct change in the presently accepted rest regimen and little or no change in dietary treatment. They are based on the conclusions of a carefully controlled study of enlisted military personnel (age range, 17 to 45, median 21) with acute infectious hepatitis. Their applicability to the more severe forms of the disease in older or less well nourished patients has not been established.

Since physical activity to the point of fatigue may be harmful, patients with acute hepatitis should be hospitalized as soon as the diagnosis is made. They should be urged to rest in bed as long as acute symptoms persist. Once they begin to feel well, regardless of the degree of jaundice, they should not be forced to stay in bed more than 1 hour after each meal. Restriction to the hospital ward, however, is essential to decrease undue activity or exertion. Allowing *ad lib.* activity on the ward (without any required exertion) circumvents the usual delay necessary for recuperation from the effects of prolonged rest in bed and appreciably shortens the period of hospitalization.

Patients so treated may be discharged from the hospital and physical reconditioning may be undertaken after the total serum bilirubin concentration (Ducci and Watson (17)) is below 1.5 mg. per 100 ml. and the bromsulphalein retention in 45 minutes below 6 per cent for a period of not less than 1 week. Patients whose bromsulphalein retention stabilizes between 5 and 10 per cent may be discharged from the hospital with safety. Those with persistently higher levels will require individual management.

Following discharge from the hospital it is well to follow all patients for 2 weeks with weekly physical examinations, serum bilirubins and bromsulphalein tests. Recurrent abnormalities will occur rarely and are probably indications for rehospitalization.

The optimal diet in the treatment of infectious hepatitis consists of about 3000 calories containing approximately 150 grams each of protein and fat. Intakes above this level should be *ad lib.* Although fried and greasy foods may cause indigestion, the fat contained in meat, eggs, and dairy products is not harmful and adds greatly to the palatability of the diet. During the stage of severe anorexia the patient should be urged to take frequent small feedings. Intravenous glucose solutions should be administered when necessary to maintain a minimal caloric fluid intake. Although the forcing of a high-protein, high-fat diet, by stomach tube if necessary, has been demonstrated to hasten recovery on the average, critically ill patients with fulminating disease or impending hepatic coma may be harmed by excess dietary protein. In these few patients, therefore, it is probably unwise to administer more than a maintenance quantity of protein.

Intravenous protein hydrolysates, plasma or blood transfusions have no place in the nutritional therapy of patients with uncomplicated infectious hepatitis.

APPENDIX ONE

Summary of Statistical Methods[24] *Used in Basic Analyses of the First and Second Studies*

A. First study

1. ***Experimental design.*** The first study was designed as a 2×2 factorial experiment in randomized blocks. Two treatment factors, diet and rest, were each represented at two levels, *"ad lib."* and "forced." Blocks of four patients each were formed from patients admitted to the hospital at about the same time, and each block comprised a complete replicate of the four treatment combinations. Duration of illness from hospital admission to convalescence was chosen as the primary measure of treatment effects.

2. ***Preliminary estimation of the size of the experiment.*** Retrospective observations on clinical records at the

[24] This summary is intended for those readers who are familiar with the applications of the theory and techniques of "analysis of variance" in biological experiments.

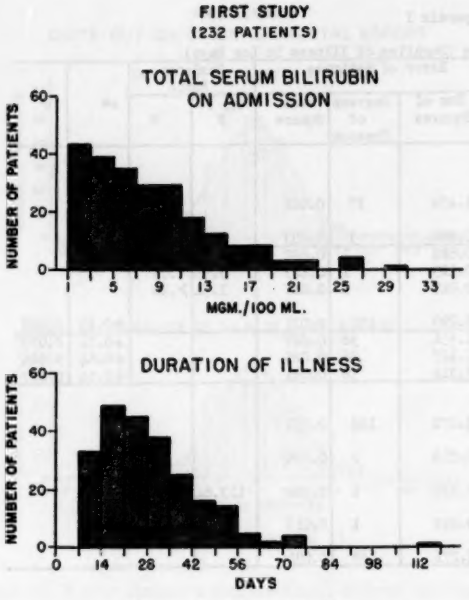

FIGURE 1 (Appendix I)

Hepatitis Center in Kyoto (see Sec. 1.2) provided information which was essential to the planning of the controlled studies. The records of 80 patients, who satisfied the criteria for admission to the first study (Sec. 2.1), were used to estimate the variability in duration of illness. This group had a mean duration of illness of 34 days and a standard deviation of ± 16 days, hence a coefficient of variation of 47 per cent. These preliminary data also demonstrated a reasonably linear relationship between the total serum bilirubin on admission and the duration of hospitalization, with a correlation coefficient of about + 0.6. By using this correlation in an analysis of covariance, it was expected that the coefficient of variation for duration of illness might be reduced to about 38 per cent.

Because of the varied clinical impressions of the investigators and others about the effects on duration of illness to be expected from the treatments under study, it seemed desirable to avoid fallacious acceptance of a null hypothesis if, in fact, a "real" treatment effect of 7 days or more might exist. Therefore, the sensitivity of the experiment was planned to detect such an effect as significant at the 5 per cent level with a Type II error of 5 per cent or less. To achieve this sensitivity in the face of the variability described above, it was estimated that at least 50 replicates or 200 patients would be required.

3. *Analyses of covariance.* Frequency distributions of the independent variable (x), admission total serum bilirubin, and the dependent variable (y), duration of illness in days, are shown in Figure 1. The analysis of covariance for these data is presented in Table I. In this analysis the differences among the regression coefficients in the "block × treatment" interaction terms were not significant. Hence, an average regression coefficient of + 1.850 was used for estimating the reduced error mean square. However, the three independent components of error variance, *i.e.*, the "block × treatment" interactions, differed significantly after reduction for regression, according to Bartlett's test (39) for homogeneity of vari-

Table I, Appendix I

First Study: Analysis of Covariance (Duration of Illness in Days)

Source of Variation	Degrees of Freedom	Sum of Squares and Products			Error of Estimate			Variance Ratio		$\bar{r}$*	b'†
		$[x^2]$	$[xy]$	$[y^2]$	Sum of Squares	Degrees of Freedom	Mean Square	F	P		
Total	231	6928.36	11811.00	54775.50							
Blocks	57	2304.40	3547.30	12577.00	7244.14	57	127.09				
Treatments	3	42.96	- 210.02	2209.81	3115.62	3	1038.54	7.26	<.001		
Diet	1	10.35	- 147.00	2088.00	2661.23	1	2661.23	18.61	<.001		
Rest	1	21.48	- 41.99	82.09	309.51	1	309.51	2.16	.10		
Diet x Rest	1	11.12	- 21.02	39.72	155.17	1	155.17	1.08			
Error	171	4581.00	8473.72	39988.69	24314.41	170	143.03			+ 0.63	1.850
Block x Diet	57	1794.00	3708.45	13455.50	5789.60	56	103.39			+ 0.76	2.067
Block x Rest	57	1461.53	2396.74	10988.41	7068.02	56	126.21			+ 0.60	1.640
Block x Diet x Rest	57	1325.47	2368.52	15534.78	11302.40	56	201.83			+ 0.52	1.787
Deviations from Individual Regressions					24160.02	168	143.81				
Differences among Individual Regressions					154.39	2	77.19				
Reduction due to Average Regression					15674.28	1	15674.28	109.59	<001		
Non-additivity					687.90	1	687.90	4.92	<05		
Remainder					23626.51	169	139.80				

* Correlation coefficient
† Regression coefficient

Table II, Appendix I

First Study: Analysis of Covariance (Duration of Illness in Log Days)

Source of Variation	Degrees of Freedom	Sum of Squares and Products			Error of Estimate			Variance Ratio		r*	b†
		$[x^2]$	$[xy]$	$[y^2]$	Sum of Squares	Degrees of Freedom	Mean Square	F	P		
Total	231	6928.356	178.440	12.197							
Blocks	57	2304.401	54.977	2.762	1.474	57	0.026				
Treatments	3	42.957	- 3.817	0.609	0.848	3	0.283	9.09	<.001		
Diet	1	10.349	- 2.372	0.544	0.682	1	0.682	21.91	<.001		
Rest	1	21.484	- 1.032	0.050	0.123	1	0.123	3.95	<.05		
Diet x Rest	1	11.123	- 0.414	0.015	0.047	1	0.047	1.51	>.20		
Error	171	4580.998	127.280	8.826	5.290	170	0.031			+0.63	0.028
Block x Diet	57	1793.996	54.128	3.164	1.531	56	0.027			+0.72	0.030
Block x Rest	57	1461.531	37.627	2.396	1.427	56	0.025			+0.64	0.026
Block x Diet x Rest	57	1325.472	35.525	3.267	2.314	56	0.041			+0.54	0.027
Deviations from Individual Regressions					5.272	168	0.031				
Differences among Individual Regressions					0.018	2	0.009				
Reduction due to Average Regression					3.536	1	3.536	113.64	<.001		
Non-additivity					0.019	1	0.019				
Remainder					5.271	169	0.031				

* Correlation coefficient
† Regression coefficient

Table III, Appendix I

First Study: Treatment Comparisons with Separate Components of Error

A. Duration of Illness in Days

Source of Variation	Degrees of Freedom	Sum of Squares and Products			Error of Estimate			Variance Ratio	
		$[x^2]$	$[xy]$	$[y^2]$	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Blocks x Diet	57	1794.00	3708.45	13455.50	5789.60	56	103.39		
Diet					2724.25	1	2724.25	26.35	<.001
Blocks x Rest	57	1461.53	2396.74	10998.41	7068.02	56	126.21		
Rest					273.57	1	273.57	2.17	>.10
Blocks x Diet x Rest	57	1325.47	2368.52	15534.78	11302.40	56	201.83		
Diet x Rest					149.12	1	149.12		

B. Duration of Illness in Log Days

Source of Variation	Degrees of Freedom	Sum of Squares and Products			Error of Estimate			Variance Ratio	
		$[x^2]$	$[xy]$	$[y^2]$	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Blocks x Diet	57	1793.996	54.128	3.164	1.531	56	0.027		
Diet					0.692	1	0.692	25.33	<.001
Blocks x Rest	57	1461.531	37.627	2.396	1.427	56	0.025		
Rest					0.115	1	0.115	4.52	<.05
Blocks x Diet x Rest	57	1325.472	35.525	3.267	2.314	56	0.041		
Diet x Rest					0.045	1	0.045	1.09	>.20

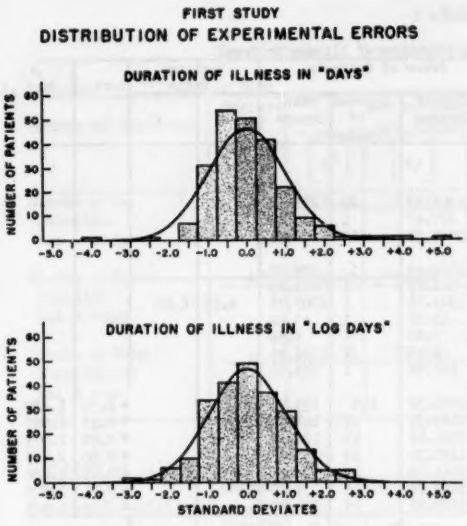

FIGURE 2 (Appendix I)

ances. Rather than use a pooled estimate of error variance for tests of significance of the treatment effects, as done in Table I, it is more appropriate in the face of heterogeneous errors to test each treatment effect against its corresponding subdivision of error variance, as is done in Table IIIA. In the present case, both procedures give similar results, although the latter test is less precise.

The discovery of error heterogeneity led to further investigation of the data with respect to the three basic assumptions involved in the "analysis of variance" under infinite models. (An infinite model is essential in the present instance because of the use of "covariance.")

a) Homogeneity of error variance—heterogeneity is present in these data by Bartlett's criterion.
b) Additivity of block and treatment effects—Tukey's test (40) for non-additivity was significant at the 5 per cent level.
c) Normal distribution of residual errors—the upper histogram in Figure 2 shows the distribution of residual errors measured in days' duration of illness with best fitting normal curve superimposed. A chi-square test for "goodness of fit" demonstrated significant departure from normality, the observed distribution being leptokurtic and positively skew.

The failure of data from the first study to satisfy the assumptions implicit in the mathematical model on which the tests of significance are based led to a change in metameter for the dependent variable from "days" to "log days." The effect of the transformation on the distribution of adjusted durations of illness is shown in Figure 3, and the effect on the distribution of residual errors is shown in Figure 2. The latter distribution on the transformed scale does not depart significantly from normal. The corresponding analysis of covariance is displayed in Table II, in which the subdivisions of error variance are now homogeneous and the test for non-additivity is not significant. There is a distinct "clinical" justification for the transformation to log days. Since any reduction in duration of illness might be proportional to the total duration, a constant percentage reduction seems more applicable than a fixed absolute reduction.

4. *Sensitivity of the experiment.* The Type II error in the first study was found to be approximately 0.01 for a Type I error of 0.05, as estimated by the use of Tang's "Tables of the Power Function of Analysis of Variance" (41).

B. Second study

1. *Experimental design.* The second study was designed as a $2 \times 2 \times 2$ factorial experiment in randomized blocks with the three-factor interaction completely confounded with blocks. Three dietary factors—total calories, percentage protein calories, and supplementary choline and vitamins—were each represented at two levels. Admission procedure and measurements of treatment effects were similar to those used in the first study.

2. *Preliminary estimate of the size of the experiment.* Estimates of variability obtained from the first study were used to predict the number of replicates required to detect a 20 per cent reduction in duration of illness with a Type I error of 0.05 and Type II error of not more than 0.05.

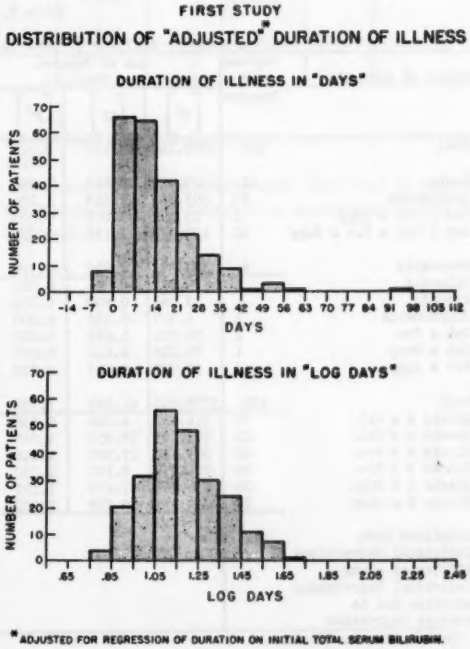
FIRST STUDY
DISTRIBUTION OF "ADJUSTED" DURATION OF ILLNESS
DURATION OF ILLNESS IN "DAYS"
NUMBER OF PATIENTS
DAYS
DURATION OF ILLNESS IN "LOG DAYS"
NUMBER OF PATIENTS
LOG DAYS
*ADJUSTED FOR REGRESSION OF DURATION ON INITIAL TOTAL SERUM BILIRUBIN.

FIGURE 3 (Appendix I)

Table IV, Appendix I

Second Study: Analysis of Covariance (Duration of Illness in Days)

Source of Variation	Degrees of Freedom	Sum of Squares and Products			Error of Estimate			Variance Ratio		r*	b†
		$[x^2]$	$[xy]$	$[y^2]$	Sum of Squares	Degrees of Freedom	Mean Square	F	P		
Total	167	5078.14	7788.54	45849.12							
Blocks	41	1105.80	1883.19	12410.12							
Replicates	20	593.04	1062.39	6181.12	4303.59	20	215.18				
Cal x Pro x Supp	1	15.42	83.02	446.88	227.47	1	227.47				
Rep x Cal x Pro x Supp	20	497.33	737.78	5782.12	4687.63	19	246.72				
Treatments	6	193.38	278.31	2069.57	1669.49	6	278.25				
Calories	1	20.37	-19.50	18.67	121.25	1	121.25				
Protein	1	3.07	67.83	1500.02	1303.76	1	1303.76	6.75	<.05		
Supplements	1	4.90	21.87	97.52	43.22	1	43.22				
Cal x Pro	1	52.93	96.55	176.10	5.86	1	5.86				
Cal x Supp	1	59.40	127.25	272.60	24.95	1	24.95				
Pro x Supp	1	52.71	-15.68	4.67	165.92	1	165.92				
Error	120	3778.96	5627.04	31369.43	22990.52	119	193.20			+0.52	1.489
Blocks A x Cal	20	514.31	559.05	3297.00	2689.31	19	141.54			+0.43	1.087
Blocks B x Cal	20	704.09	1386.88	5856.67	3124.86	19	164.47			+0.68	1.973
Blocks A x Pro	20	603.14	1246.70	7047.24	4470.30	19	235.28			+0.60	2.067
Blocks B x Pro	20	501.10	711.77	4655.14	3644.12	19	191.80			+0.47	1.420
Blocks A x Supp	20	431.97	346.58	4827.14	4549.07	19	239.42			+0.24	0.802
Blocks B x Supp	20	1024.36	1376.06	5686.24	3837.72	19	201.99			+0.58	1.343
Deviations from Individual Regressions					22315.40	114	195.75				
Differences among Individual Regressions					675.12	5	135.02				
Reduction due to Average Regression					8378.91	1	8378.91	43.37	<.001		

* Correlation coefficient

† Regression coefficient

Table V, Appendix I

Second Study: Analysis of Covariance (Duration of Illness in Log Days)

Source of Variation	Degrees of Freedom	Sum of Squares and Products			Error of Estimate			Variance Ratio		r*	b†
		$[x^2]$	$[xy]$	$[y^2]$	Sum of Squares	Degrees of Freedom	Mean Square	F	P		
Total	167	5078.140	113.973	8.512							
Blocks	41	1105.797	29.846	2.204							
Replicates	20	593.041	17.618	1.296	0.787	20	0.039	1.26	>.20		
Cal x Pro x Supp	1	15.421	1.073	0.075	0.033	1	0.033	1.06	>.20		
Rep x Cal x Pro x Supp	20	497.335	11.155	0.834	0.584	19	0.031				
Treatments	6	193.384	2.939	0.320	0.283	6	0.047				
Calories	1	20.371	-0.286	0.004	0.026	1	0.026				
Protein	1	3.067	0.800	0.209	0.176	1	0.176	4.92	<.05		
Supplements	1	4.903	-0.020	0.000	0.003	1	0.003				
Cal x Pro	1	52.931	1.033	0.020	0.000	1	0.000				
Cal x Supp	1	59.405	2.141	0.077	0.012	1	0.012				
Pro x Supp	1	52.707	-0.728	0.010	0.065	1	0.065	1.82	>.10		
Error	120	3778.958	81.189	5.988	4.244	119	0.036			+0.54	0.021
Blocks A x Cal	20	514.307	8.506	0.558	0.417	19	0.022			+0.50	0.017
Blocks B x Cal	20	704.093	18.859	1.067	0.562	19	0.030			+0.69	0.027
Blocks A x Pro	20	603.138	17.375	1.446	0.945	19	0.050			+0.59	0.029
Blocks B x Pro	20	501.097	9.182	0.750	0.581	19	0.031			+0.46	0.018
Blocks A x Supp	20	431.966	7.499	1.105	0.975	19	0.051			+0.33	0.017
Blocks B x Supp	20	1024.357	19.768	1.062	0.681	19	0.036			+0.60	0.019
Deviations from Individual Regressions					4.162	114	0.037				
Differences among Individual Regressions					0.082	5	0.016				
Reduction due to Average Regression					1.744	1	1.744	48.91	<.001		

* Correlation coefficient

† Regression coefficient

Table VI, Appendix I

Second Study: Treatment Comparisons with Separate Components of Error

A. Duration of Illness in Days

Source of Variation	Degrees of Freedom	Sum of Squares and Products			Error of Estimate			Variance Ratio	
		$[x^2]$	$[xy]$	$[y^2]$	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Blocks x Cal	40	1218.40	1945.93	9153.67	6045.77	39	155.02		
Calories					130.73	1	130.73		
Pro x Supp					181.36	1	181.36	1.17	>.20
Blocks x Pro	40	1104.24	1958.47	11702.38	8228.85	39	211.00		
Protein					1265.55	1	1265.55	6.00	<.05
Cal x Supp					7.66	1	7.66		
Blocks x Supp	40	1456.32	1722.64	10513.38	8475.75	39	217.33		
Supplements					52.48	1	52.48		
Cal x Pro					20.99	1	20.99		

B. Duration of Illness in Log Days

Source of Variation	Degrees of Freedom	Sum of Squares and Products			Error of Estimate			Variance Ratio	
		$[x^2]$	$[xy]$	$[y^2]$	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Blocks x Cal	40	1218.400	27.364	1.625	1.011	39	0.026		
Calories					0.027	1	0.027	1.03	>.20
Pro x Supp					0.071	1	0.071	2.73	>.10
Blocks x Pro	40	1104.235	26.557	2.195	1.556	39	0.040		
Protein					0.171	1	0.171	4.30	<.05
Cal x Supp					0.008	1	0.008		
Blocks x Supp	40	1456.323	27.267	2.167	1.657	39	0.042		
Supplements					0.003	1	0.003		
Cal x Pro					0.000	1	0.000		

3. *Analysis of covariance.* Tables IV, V, and VI present the analyses of covariance for both the original and transformed measurements of duration of illness. The analysis suggests that no reduction in error variance was achieved by restricting the size of block to four patients, since the mean square for "Replicate × Cal × Pro × Supp interaction" does not significantly exceed the error variance.

4. *Sensitivity of the experiment.* The Type II error in the second study was found to be approximately 0.05 for a Type I error of 0.05.

APPENDIX II

Supplementary Tables

Table 1, Appendix II

Distribution of Liver Function Tests in 279 "Normal" Soldiers - Kyoto and Sasebo Series

Total Serum Bilirubin					Thymol Turbidity				
		Kyoto Series History		Sasebo			Kyoto Series History		Sasebo
mgs/100 ml	Normal	Equivocal	Total	Series	Units	Normal	Equivocal	Total	Series
.10- .19	0	1	1	77	.5	1	3	4	0
.20- .29	5	5	10	42	1.0	17	3	20	23
.30- .39	22	9	31	80	1.5	5	4	9	79
.40- .49	37	7	44	139	2.0	56	18	74	44
.50- .59	34	17	51	88	2.5	30	10	40	155
.60- .69	34	15	49	49	3.0	29	12	41	81
.70- .79	27	6	33	24	3.5	27	10	37	28
.80- .89	16	3	19	15	4.0	13	3	16	19
.90- .99	15	8	23	8	4.5	13	1	14	15
1.00-1.09	8	2	10	4	5.0	4	0	4	7
1.10-1.19	3	0	3	0	5.5	7	3	10	4
1.20-1.29	1	0	1	2	6.0	0	0	0	0
1.30-1.39	1	0	1	2	6.5	3	5	8	2
1.40-1.49	0	0	0	0	7.0	0	0	0	1
1.50-1.59	0	0	0	0	7.5	0	0	0	0
1.60-1.69	1	0	1	0	8.0	0	0	0	1
1.70-1.79	1	0	1	0	8.5	0	0	0	0
1.80-2.49	0	0	0	0	9.0	1	0	1	1
2.50-2.59	1	0	1	0	9.5-11.5	0	0	0	0
					12.0	0	1	1	
	206	73	279	460		206	73	279	460

Prompt Direct Serum Bilirubin					Zinc Turbidity				
.02-.03	2	3	5	21	2	1	2	3	
.04-.05	11	5	16	48	3	8	6	14	
.06-.07	25	12	37	108	4	22	5	27	
.08-.09	33	13	46	90	5	19	7	26	
.10-.11	39	12	51	84	6	55	17	72	
.12-.13	33	9	42	68	7	38	15	53	
.14-.15	22	10	32	23	8	21	4	25	
.16-.17	21	2	23	11	9	19	2	21	
.18-.19	11	2	13	3	10	10	4	14	
.20-.21	5	4	9	0	11	5	5	10	
.22-.23	1	0	1	2	12	3	4	7	
.24-.25	1	0	1	1	13	2	2	4	
.26-.27	2	0	2	1	14	1	0	1	
.28-.29	0	0	0		15-17	0	0	0	
.30-.31	0	1	1		18	0	1	1	
	206	73	279	460		204	74	278	

Δ pH/hr.	Cholinesterase			Thymol Flocculation				
.40- .49	5	1	6	0	132	49	181	410
.50- .59	5	2	7	1+	70	21	91	34
.60- .69	46	11	57	2+	3	2	5	14
.70- .79	49	18	67	3+	0	1	1	2
.80- .89	33	19	52	4+	1	0	1	0
.90- .99	45	17	62		206	73	279	460
1.00-1.09	16	2	18					
1.10-1.19	3	2	5	Cephalin Flocculation				
	202	72	274					
				0	193	65	258	413
				1+	4	4	8	43
				2+	8	4	12	3
				3+	1	0	1	0
				4+	0	0	0	1
					206	73	279	460

Table II, Appendix II

Sample Diet Check Sheet: Forced Diet Wards, First Study

FOOD		SECONDS	REFUSALS	TOTAL	CHO	PRO	FAT
Amount	Weight						
1 cup Chilled Tomato Juice	(150)			1	6	2	
2 Poached Eggs	(100)			1		12	12
1 slice Toast	(30)			1	16	3	1
1 pat Butter	(8)			1			7
1 tablespoon Jam	(20)			1	13		
Evaporated Milk	(15)			1	2	1	1
1 shaker sugar	(1t)			1	5		
1 carton Milk	(240)			1	12	8	9
	TOTAL				54	26	30
Special Eggnog	(260)			1	21	21	13
1 teaspoon Sugar	(5)			1	5		
2 servings Baked Chicken - 180	(190)		40	150		42	14
1 dipper Mashed Potatoes	(60)			1	10	1	4
1 serving Baked Tomatoes	(100)			40	2		
1 spoon Old-Fashioned Coleslaw	(40)		all				
1 serving Corn Bread	(80)			1	38	6	7
1/2 pat Butter	(4)			1			3
1 spoon Lemon Meringue Pudding	(80)			1	34	2	2
Evaporated Milk	(15)			1	2	1	1
1 shaker sugar	(1t)			1	5		
1 carton Milk	(240)			1	12	8	9
	TOTAL				183	107	83
Special Eggnog Plus	(200)			1	16	16	10
Ice Cream	(75)			1	16	3	10
2 pieces Steak - 150	(150)			150		41	20
1 spoon Scalloped Potatoes	(80)			1	12	2	5
1 spoon Buttered Peas	(60)			1	5	2	
1 spoon Mixed Vegetable Salad	(40)		all				
1 teaspoon Oil & Vinegar	(5)		all				
1/2 slice Bread	(15)			1	8	2	1
1/2 pat Butter	(4)			1			3
1 spoon Apple Crisp	(80)			1	24	6	2
Evaporated Milk	(15)			1	2	1	1
1 shaker sugar	(1t)			1	5		
1 carton Milk	(240)	1		2	24	16	18
	TOTAL				295	196	153
Special Eggnog plus	(200)			1	16	16	10
Ice Cream	(75)			1	16	3	10
1 slice Bread	(30)		all				
1/2 pat Butter } Sandwich	(4)		all				
60 Roast Beef	(60)		all				
	TOTAL GRAMS				327	215	173

Total Calories: 3725
Total % Protein Calories: 23.1%

Table III, Appendix II

Sample Diet Check Sheet: Ad Lib Wards, First Study

FOOD		SECONDS	REFUSALS	TOTAL	CHO	PRO	FAT
Amount	Weight						
1 cup Chilled Tomato Juice	(150)		1/2	75	3	1	
1 box Assorted Dry Cereal Puff Wheat	(15)			1	11	2	
1 Poached Egg on	(50)			1		6	6
1 slice Toast	(30)			1	16	3	1
1 slice Toast	(30)			1	16	3	1
1 pat Butter	(8)			1			7
1 tablespoon Jam	(20)			1	13		
Evaporated Milk	(15)			1	2	1	1
1 shaker sugar	(2t)			2	10		
1 carton Milk	(240)			1	12	8	9
	TOTAL				83	24	25
1 cup Fruit Juice - Orange	(150)			1	18	1	
1 ladle Bean Soup	(180)		all				
1 tablespoon Croutons	(10)		all				
1 serving Fried Fish	(65)			65	5	13	8
1 tablespoon Tartar Sauce	(20)			1			10
1 dipper Mashed Potatoes	(120)		all				
1 serving Green Beans	(60)			1	3		
1 spoon Old-Fashioned Coleslaw	(30)			1	2		3
1 serving Corn Bread	(80)				38	6	7
1 pat Butter	(8)			1			7
1 spoon Lemon Meringue Pudding	(120)			1	52	2	2
Evaporated Milk	(15)		all				
1 shaker sugar	(2t)			2	10		
1 carton Milk	(240)			1	12	8	9
	TOTAL				223	54	71
1 cup Fruit Juice	(150)						
1 ladle Salmon Chowder	(150)		all				
1 Cracker	(10)		all				
1 piece Grilled Steak	(80)	70	20	130		35	17
1 spoon Scalloped Potatoes	(100)			1	15	3	6
1 spoon Buttered Peas	(40)			1	5	2	
1 spoon Mixed Vegetable Salad	(35)		all				
1 teaspoon Oil & Vinegar	(5)		all				
1 slice Bread	(30)			1	16	3	1
1 pat Butter	(8)			1			7
1 serving Turnover	(80)	1		2	54	5	21
Evaporated Milk	(15)		all				
1 shaker sugar	(2t)			2	10		
1 carton Milk	(240)			1	12	8	9
Lemon Sauce	(10)	1		2	10		
1 small Strawberry Sundae	( )				67	6	18
1 cup Fruit Juice	(150)				18	1	
	TOTAL GRAMS				430	117	150

Total Calories: 3538
Total % Protein Calories: 13.2%

Table IV, Appendix II

Accuracy of the Dietary Calculations

A. Comparison of mean data obtained by calculation (aides), weighing (dietitians), and chemical analysis.

		Calculated by the aides	Weighed by the dietitians	Analyzed by the chemists
First Study				
Forced Diets (4 days averaged)	CHO-gm.	308	306	306*
	Pro "	189	190	205
	Fat "	168	161	134
	Calories	3504	3427	3253
Ad Lib Diets (4 days averaged)	CHO-gm.	367	366	370*
	Pro "	119	122	136
	Fat "	130	123	106
	Calories	3120	3055	2981
Second Study				
Ward 23 High calorie High protein (3 days averaged)	CHO-gm.	357	357	339*
	Pro "	185	181	196
	Fat "	184	182	144
	Calories	3821	3795	3433
Ward 41 Normal calorie Normal protein (3 days averaged)	CHO-gm.	311	299	291*
	Pro "	71	68	80
	Fat "	105	107	90
	Calories	2469	2458	2293

B. Rank correlations between calculated and chemically analyzed whole days' diets.

Study	Number of diets	Rank correlation coefficient Protein	Fat
First	8	.79	.96
Second	6	.67	.80

C. Mean differences between methods.

Comparison		Mean difference in Grams	S.E.	P
Protein -	Calculated minus analyzed	-14.	6.6	.05
	Calculated minus weighed	+ 1.	1.7	.6
Fat -	Calculated minus analyzed	+28.	4.2	.001
	Calculated minus weighed	+ 4.	1.5	.02

* Calculated from the difference between the sum of the protein and fat calories and the total calories.

Table V, Appendix II

Distributions of Clinical and Laboratory Findings on Admission by First Study Treatment Groups, and Comparisons of First and Second Studies

Treatment Regimens*	DR	Dr	dR	dr	P	First Study Mean	First Study S.D.	2nd Study Mean	2nd Study S.D.	P
Quantitative Variables										
"Doctor Effect" Improbable										
1. Age in years	23	23	24	23	.4	23	3.7	23	3.9	.3
2. Duration of hospitalization prior to Kyoto admission in days	4	4	4	3	.2	4	2.7	4	2.1	.4
3. Weight in pounds on admission	149	155	150	148	.08	150	21.6	152	19.1	.3
4. Admission total serum bilirubin mgm./100 ml.	8.29	8.10	7.46	8.50	.9	8.07	5.6	8.32	5.4	.6
5. Admission prompt direct serum bilirubin mgm./100 ml.	4.13	3.91	3.71	4.11	.9	3.96	2.8	4.70	3.2	.01
6. Admission thymol turbidity in units	10.1	10.3	9.6	9.4	.8	9.6	4.7	8.4	5.1	.001
7. Admission zinc turbidity in units	12.0	13.6	9.6	12.8	.1	12.6	4.5	9.8	4.6	.001
"Doctor Effect" Possible										
8. Loss of weight between arrival in Korea and admission, in pounds	13	12	12	13	.9	12.5	9.9	10.9	8.4	.09
9. Duration of symptoms before admission	11	10	10	11	.7	10	4.6	12	5.4	.01
10. Duration of icterus before admission	7	8	7	8	.9	7.5	4.0	8	4.2	.02
11. Number of days before admission on which patient ate one meal or less per day	8	8	9	8	.5	8	4.3	8	4.8	.8
12. Number of days from onset of symptoms to hospitalization in which the patient's activity was more than 1/3 of normal	6	5	5	7	.02	6	4.4	7	5.5	.001
13. Same from onset of jaundice to hospitalization	4	3	3	4	.2	3	3.4	5	3.8	.5
Qualitative Variables										
"Doctor Effect" Improbable										
1. Race - % white	91	83	86	81	.4	85		81		.3
2. Source of infection - % Korea	95	97	98	91	.2	94		93		.7
3. % with past history of liver disease	12	10	2	8	.1	8		8		--
4. Cephalin flocculation - % positive on admission	69	69	71	69	.9	69		37		.001
5. Thymol flocculation - % positive on admission	52	60	48	52	.5	53		34		.001
"Doctor Effect" Possible										
History in 120 days before admission										
6. % with other diseases	35	56	49	42	.1	45		46		.8
7. % with subnormal food intake	9	19	14	27	.06	17		16		.8
8. % with active physical exertion	6	38	24	20	.001	22		14		.03
9. % with fatigue	5	10	29	20	.002	16		13		.4
10. % receiving parenteral injections	95	94	79	78	.003	87		88		.7
11. % with moderate to severe alcohol consumption	47	32	40	13	.008	33		45		.01
Present Illness										
12. % with 2 or more severe symptoms	37	44	52	34	.03	42		32		.03
13. % with food intake decreasing significantly in the 3 days before admission when compared with the previous 3 days	20	21	8	21	.002	17		14		.4
14. % with anorexia on admission	74	68	71	69	.9	71		79		.5
15. % with malaise on admission	52	70	70	33	.003	56		71		.001
16. % with liver pain on admission	42	62	49	44	.09	49		50		.8
17. % with nausea on admission	46	41	30	31	.2	37		50		.007
18. % with vomiting on admission	12	8	6	5	.4	8		18		.002
19. % with moderate hepatomegaly on admission	46	48	26	47	.03	42		46		.4
20. % with marked hepatomegaly on admission	40	34	61	53	.01	47		46		.8
21. % with hepatic tenderness on admission	68	69	73	75	.8	71		63		.07
Total Number of Patients	65	64	63	65		257		189		

*See footnote to Table VII, Appendix II for explanation of symbols referring to treatment regimens.

Table VI, Appendix II

Distributions of Clinical and Laboratory Findings on Admission by Treatment Groups in the Second and Third Studies

Treatment Regimens:	CPS	CPs	cPS	cPs	CpS	Cps	cpS	cps	P*	E+	e+	P
Total patients	24	23	24	23	24	24	23	24	—	95	94	
Quantitative Variables												
"Doctor Effect" Improbable												
1. Age in years	24	22	25	22	24	24	23	24	.4	23	23	—
2. Duration of hospitalization prior to Kyoto admission in days	4	5	3	4	4	4	3	5	.3	4	4	—
3. Weight in pounds on admission	154	151	151	152	146	155	154	153	.9	153	151	.6
4. Admission total serum bilirubin mgm./100 ml.	8.58	7.90	9.34	8.37	7.46	10.46	7.45	7.02	.6	8.95	7.96	.2
5. Admission prompt direct serum bilirubin mgm./100 ml.	4.54	4.40	4.98	4.42	4.36	6.31	4.33	4.11	.1	5.02	4.37	.2
6. Admission thymol turbidity in units	9.6	7.5	7.4	7.7	8.1	8.9	8.0	9.9	.9	8.0	8.5	.3
7. Admission zinc turbidity in units	10.5	9.8	9.1	9.3	9.1	9.9	10.6	11.0	.9	10	10	—
8. Admission bromsulphalein retention, % in 45 minutes	48	46	44	46	42	50	50	40	.06	48	43	.02
9. Admission cholinesterase, pH/hr.	.69	.65	.67	.68	.61	.58	.65	.62	.5			
"Doctor Effect" Possible												
10. Loss of weight between arrival in Korea and admission, in pounds	9	9	10	9	12	10	14	13	.3	10	11	.5
11. Duration of symptoms before admission	12	14	12	10	13	10	12	13	.3	12	12	—
12. Duration of icterus before admission	7	10	9	9	9	7	8	9	.3	9	9	—
13. Number of days before admission on which patient ate one meal or less per day	10	11	7	6	10	8	7	8	.01	8	8	—
14. Number of days from onset of symptoms to hospitalization in which the patient's activity was more than 1/3 of normal	8	9	8	6	5	4	9	10	.01	7	7	—
15. Same from onset of jaundice to hospitalization	4	7	6	5	4	2	5	6	.01	5	5	—

Qualitative Variables	CP	cP	Cp	cp	P**	S	s	P#			
"Doctor Effect" Improbable											
1. Race - % white	72	89	90	74	.04	84	79	.4	78	85	.2
2. Source of infection -% Korea	98	91	90	94	.4	94	93	.8	93	94	.8
3. % with past history of liver disease	9	9	10	6	.9	8	10	.6	6	11	.3
4. Cephalin flocculation - % positive on admission	33	36	40	43	.9	35	41	.4	35	41	.4
5. Thymol flocculation - % positive on admission	28	35	35	38	.8	35	33	.7	27	41	.05
""Doctor Effect" Possible											
History in 120 days before admission											
6. % with other diseases	49	38	42	53	.4	46	45	.9	51	40	.2
7. % with subnormal food intake	21	19	15	12	.6	17	15	.8	17	15	.7
8. % with active physical exertion	6	19	13	17	.004	11	17	.4	13	15	.7
9. % with fatigue	20	7	28	2	.002	14	15	.8	18	10	.1
10. % receiving parenteral injections	94	77	85	98	.008	87	89	.9	91	86	.3
11. % with moderate to severe alcohol consumption	31	49	46	53	.06	41	47	.5	43	46	.7
Present Illness											
12. % with 2 or more severe symptoms	62	21	19	26	.01	33	31	.8			
13. % with food intake decreasing significantly in the 3 days before admission when compared with the previous 3 days	23	6	10	15	.04	13	13	—			
14. % with anorexia on admission	92	74	88	64	.003	82	77	.5			
15. % with malaise on admission	89	72	73	49	.003	73	69	.6			
16. % with liver pain on admission	64	55	44	38	.06	43	57	.06			
17. % with nausea on admission	62	53	44	40	.2	50	50	—			
18. % with vomiting on admission	23	17	15	17	.7	14	22	.1			
19. % with moderate hepatomegaly on admission	28	60	38	60	.003	45	47	.8			
20. % with marked hepatomegaly on admission	70	32	52	30	.003	46	46	—			
21. % with hepatic tenderness on admission	81	74	85	13	.001	63	64	.9			

* Probability values for differences among the 8 treatment groups.
** Probability values for difference between wards.
\# Probability values for differences within wards.

\+ E = early exercise group.
e = late exercise group. See footnote to Table VII for explanation of other treatment symbols.

Table VII, Appendix II

Patients Removed from Studies in Basic Analyses

Treatment groups*		Reason for removal	Admission total serum bilirubin	Start of convalescence
		A. FIRST STUDY		
1	dR	Clinical infectious mononucleosis with rising and falling heterophile agglutination and many atypical cells on smear.	1.4	14
2	dR	Lymphogranuloma venereum. Liver function tests normal.	0.9	7
3	Dr	No symptoms, signs, or laboratory findings of hepatitis. Malingering suspected.	0.3	7
4	dr	Committed for acute psychosis on 48th day. TSB normal. BSP 7%.	19.4	51 (earliest) 58 probable
5	Dr	Emergency leave day 32, BSP 2, TSB 1.3 Needed one more normal TSB.	14.5	32 - 35
6	dR	True ambulation day unknown due to possible mix-up of bloods. Either 43 days or 10 days plus 33-day relapse.	6.7	10 or 43
7	dR	Same as #6. Either 102 days or 29 plus 73-day relapse.	8.7	29 or 102
		B. SECOND STUDY		
1	cPs	E. Histolytica in stools. Persistent fever and localized hepatic tenderness. Treated with chloraquin and terramycin. Dropped from observation.	17.7	unknown
2	CPs	E. Histolytica in stools. Severe localized hepatic tenderness. Treated with chloraquin and terramycin.	12.0	27
3	cpS	Heterophile positive. May have had hepatitis but TSB remained around 3 mg% with normal PDSB and BSP until discharge 78 days after admission. No reticulocytosis.	3.3	78
4	cps	Last replicate of 8 removed to close study.	6.9	22
5	cPs	Same	5.1	33
6	CPS	Same	2.4	30
7	CpS	Same. Dietary figures for only 6 days.	1.1	35
8	CPs	Same	5.8	38
9	cPS	Same. BSP still 6 when study ended.	15.6	62
10	cpS	Same. BSP still 7 when study ended.	5.9	61
11	Cps	Same	17.5	47

*In this and following tables where treatment groups are indicated by code, the capital letter indicates the forced regimen or high level of dietary intake and the small letter the ad lib regimen or "normal" dietary intake. Thus:

First Study D = forced diet; d = ad lib diet; R = forced rest; r = ad lib rest

Second Study P = high protein; p = "normal" protein; C = high calorie; c = "normal" calorie; S = choline and vitamin supplements; s = placebos

Table VIII, Appendix II. The effect of treatment on the incidence of patients whose serum bilirubin levels rose after admission.

	First Study						Second Study								
	Diet			Rest			Protein			Calories			Supplements		
	Forced	Ad Lib.	P	Strict	Ad Lib.	P	High	"Normal"	P	High	"Normal"	P	Added	Placebos	P
Total serum Bilirubin															
Total Patients	129	128		128	129		94	95		95	94		95	94	
Number rising*	5	18		11	12		3	17		12	8		5	15	
% rising	4	14	.01	9	9	-	3	18	.001	13	9	.4	5	16	.02
Prompt Direct Serum Bilirubin															
Total Patients	129	128		128	129		94	95		95	94		95	94	
Number rising**	5	16		10	11		3	13		9	7		5	15	
% rising	4	12	.01	8	8	-	3	14	.01	10	7	.6	5	16	.02

* By 1 mg. per 100 ml. or more **By 0.5 mg. per 100 ml. or more

Table IX. Thymol and zinc turbidities: Mean admission, convalescent and discharge values.

	First Study										Second Study*									
	Diet					Rest					Protein				Calories				Supplements			
	Forced		Ad Lib.			Forced		Ad Lib.			High		"Normal"		High		"Normal"		High		"Normal"	
	Mean	S.D.	Mean	S.D.	P	Mean	S.D.	Mean	S.D.	P	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Thymol Turb.																						
Admission	9.8	4.9	9.4	4.5	.5	9.5	4.5	9.8	4.7	.6	8.0	5.1	8.7	5.2	8.5	5.2	8.2	5.1	8.3	4.7	8.5	5.4
Convalescence	7.1	3.5	5.9	3.2	.005	6.3	3.3	6.8	3.5	.1	4.5	2.7	4.7	3.0	4.8	3.1	4.3	2.6	4.6	2.8	4.5	2.9
Discharge	4.5	2.6	4.3	2.5	.5	4.1	2.5	4.6	2.8	.1	3.4	1.6	3.3	2.0	3.5	2.0	3.2	1.7	3.4	1.9	3.3	1.7
Zinc Turb.																						
Admission	12.9	4.5	12.3	3.3	.3	12.0	4.4	13.2	3.5	.05	9.6	4.9	10.0	4.3	9.8	4.4	9.9	4.5	9.7	4.1	10.0	4.8
Convalescence	12.0	4.4	10.1	4.0	.002	10.6	3.8	11.6	4.7	.1	7.8	4.1	7.3	1.7	7.9	2.2	7.2	3.7	7.3	4.1	7.8	1.4
Discharge	9.1	3.6	8.9	3.6	.7	8.6	3.4	9.3	4.0	.1	6.8	3.1	6.9	3.2	7.2	2.9	6.6	3.1	6.6	3.4	7.0	3.1
Total Patients	129		128			128		129			94		95		95		94		95		94	

*None of the differences between treatment groups were significant

Table X, Appendix II The percentage of patients with abnormal flocculation and turbidity tests

	First Study (257 Patients)				Second Study (189 Patients)					
	Diet		Rest		Protein		Calories		Supplements	
Test and Time Performed	Forced %	Ad Lib %	Forced %	Ad Lib %	High %	"Normal" %	High %	"Normal" %	Added %	Placebos %
Cephalin Flocculation										
Admission	69	68	69	68	34	41	36	39	34	40
Highest	82	84	83	88	41	50	42	50	41	51
Convalescence	24	15	18	21	1*	9*	4	6	3	7
Discharge	6*	1*	2	4	0	0	0	0	0	0
Thymol Flocculation										
Admission	55	50	49	55	32	37	32	37	35	33
Highest	63	55	57	61	38	46	40	45	43	41
Convalescence	43	38	40	41	15	21	19	17	15	21
Discharge	24	27	22	29	8	16	14	10	12	12
Thymol Turbidity										
Admission	71	70	71	70	50	56	51	55	54	52
Highest	76	74	75	75	54	58	56	56	54	58
Convalescence	49	38	42	46	14	20	17	17	16	18
Discharge	17	14	12	18	7	7	7	6	6	7
Zinc Turbidity										
Admission	42	37	35	43	20	22	18	24	19	22
Highest	59	46	46	59	28	24	25	31	27	29
Convalescence	37*	14*	18	24	9	5	9	5	8	6
Discharge	14	11	9	16	2	5	4	3	3	4
Mean Duration of:**										
Acute illness	25	32	30	28	28	34	31	32	31	32
Observation	62	65	64	63	49	54	50	54	50	56

* Differences significant at the 5% level
** Arithmetic means

Table II, Appendix II

Comparison of flocculation and turbidity tests in first and second studies

	% of patients with abnormal tests			Mean number of units		
	1st study	2nd study	P	1st study	2nd study	P
Cephalin flocculation						
Admission	69	37	.001			
Highest	83	46	.001			
Convalescence	20	5	.001			
Discharge	3	0	.01			
Thymol flocculation						
Admission	52	34	.001			
Highest	59	42	.001			
Convalescence	41	18	.001			
Discharge	25	12	.001			
Thymol turbidity						
Admission	71	53	.001	9.5	8.5	.001
Highest	75	67	.07			
Convalescence	44	17	.001	5.5	4.5	.002
Discharge	15	7	.007	4.5	3.5	.001
Zinc turbidity						
Admission	39	21	.001	13	10	.001
Highest	53	28	.001			
Convalescence	21	7	.001	11	8	.001
Discharge	13	4	.001	9	7	.001
Total Patients	257	189		257	189	
Mean duration of acute illness				29 days	31 days	
Mean duration of observation				64 "	52 "	

Table XII

Mean eosinophil counts (first study) and cholinesterase values (second study) during the first two weeks of hospitalization.

	Eosinophil counts (number per ml)				Cholinesterase (Δ pH per hour)			
	Diet		Rest		Protein		Calories	
	Forced	Ad Lib	Forced	Ad Lib	High	"Normal"	High	"Normal"
Admission	91	95	103	88	.68	.61	.63	.65
Day 3-6	119	100	100	90	.65	.63	.65	.63
Day 7-10	104	115	119	104	.72	.65	.70	.67
Day 11-14	118	134	144	116	.74	.72	.72	.74
Convalescence					.84	.78	.81	.81
Discharge					.85	.82	.85	.82
Number of Patients	37	43	30	50	93	92	93	92

Table XIII, Appendix II

Effects of treatment on symptoms during the first three weeks of hospitalization

	First Study				Second Study					
	Diet		Rest		Protein		Calories		Supplements	
	Forced	Ad lib.	Forced	Ad lib.	High	"Normal"	High	"Normal"	Added	Placebos
Anorexia										
Admission										
Number of patients	128	127	128	127	94	95	95	94	95	94
% with symptoms	71	70	73	68	83	76	90	69	82	77
% with severe symptoms	9	14	10	13	27	15	35	6	21	20
7 - 8 days										
Number of patients	127	127	128	126	94	95	95	94	95	94
% with symptoms	16	20	25	11	26	26	35	17	28	23
% with severe symptoms	0	2	2	0	0	1	1	0	0	1
13 - 15 days										
Number of patients	110	116	117	109	88	90	87	91	91	87
% with symptoms	7	10	9	8	9	10	14	6	9	10
% with severe symptoms	1	1	1	1	0	1	0	1	0	1
20 - 22 days										
Number of patients	76	98	91	83	67	74	70	71	73	68
% with symptoms	3	3	3	2	11	5	10	6	4	12
% with severe symptoms	0	0	0	0	2	0	1	0	0	2
Malaise										
Admission										
Number of patients	128	127	128	127	94	95	95	94	95	94
% with symptoms	61	51	61	51	81	61	81	61	73	69
% with severe symptoms	24	12	23	13	29	14	35	7	24	18
7 - 8 days										
Number of patients	127	127	128	126	94	95	95	94	95	94
% with symptoms	13	24	25	11	17	27	34	11	21	23
% with severe symptoms	6	3	8	1	3	0	3	0	2	1
13 - 15 days										
Number of patients	110	116	117	109	88	90	87	91	91	87
% with symptoms	9	14	17	6	8	10	12	7	6	13
% with severe symptoms	0	3	3	0	0	0	0	0	0	0
20 - 22 days										
Number of patients	76	98	91	83	67	74	70	71	73	68
% with symptoms	1	6	7	1	10	14	14	10	8	16
% with severe symptoms	0	0	0	0	0	0	0	0	0	0
Liver Pain										
Admission										
Number of patients	128	127	128	127	94	95	95	94	95	94
% with symptoms	52	46	45	53	60	41	54	47	43	57
% with severe symptoms	16	22	24	14	18	8	17	10	15	12
7 - 8 days										
Number of patients	127	127	128	126	94	95	95	94	95	94
% with symptoms	12	11	10	13	12	8	10	10	13	7
% with severe symptoms	1	4	1	3	3	0	2	1	3	0
13 - 15 days										
Number of patients	110	116	117	109	88	90	87	91	91	87
% with symptoms	5	4	4	5	3	4	2	6	6	2
% with severe symptoms	0	3	1	2	1	0	0	1	1	0
20-- 22 days										
Number of patients	76	98	91	83	67	74	70	71	73	68
% with symptoms	3	3	3	2	2	4	2	4	3	3
% with severe symptoms	0	0	0	0	0	0	0	0	0	0
Nausea and Vomiting										
Admission										
Number of patients	128	127	128	127	94	95	95	94	95	94
% with nausea	44	31	38	36	57	42	53	47	50	50
% with vomiting	10	6	9	6	20	16	19	17	14	22
7 - 8 days										
Number of patients	127	127	128	126	94	95	95	94	95	94
% with nausea	3	8	9	2	10	7	10	7	6	11
% with vomiting	1	0	1	0	0	3	3	0	1	2
13 - 15 days										
Number of patients	110	116	117	109	88	90	87	91	91	87
% with nausea	1	5	4	2	3	2	3	2	1	5
% with vomiting	0	2	1	1	0	1	0	1	1	1
20 - 22 days										
Number of patients	76	98	91	83	67	74	70	71	73	68
% with nausea	0	3	2	1	6	3	4	4	3	6
% with vomiting,	0	1	1	0	0	1	0	1	0	2

Table XIV, Appendix II The effects of treatment on the frequency of hepatomegaly liver tenderness, spider angiomata and splenomegaly, and on weight gain during the first 3 hospital weeks

	First Study (257 Patients)				Second Study (189 Patients)					
	Diet		Rest		Protein		Calories		Supplements	
	Forced %	Ad Lib %	Forced %	Ad Lib %	High %	"Normal" %	High %	"Normal" %	Added %	Placebos %
Admission										
No enlargement	15	6	13	8	5	11	6	9	9	7
3-5 cm.	48	37	37	48	44	48	33	60	45	47
>5 cm.	37	57	50	44	51	41	61	31	46	46
Tenderness	68	74	70	72	78	49	83	44	63	64
7-9 Days										
No enlargement	12	10	12	10	8	21	9	20	11	19
3-5 cm.	63	38	45	56	53	58	47	64	60	51
>5 cm.	25	52	43	34	39	21	44	16	29	30
Tenderness	25	38	31	31	32	33	46	18	34	29
14-16 Days										
No enlargement	30	10	23	17	18	52	17	52	28	36
3-5 cm.	59	42	41	60	58	41	56	43	59	45
>5 cm.	11	48	36	23	24	7	27	5	13	19
Tenderness	8	21	15	14	12	14	19	6	15	10
21-23 Days										
No enlargement	42	19	33	28	29	67	36	59	44	52
3-5 cm.	50	48	47	52	54	25	46	33	45	33
>5 cm.	8	33	20	20	17	8	18	8	11	15
Tenderness	5	13	11	7	7	30	33	15	8	11
Splenomegaly	10	18	10	18	23	5	14	15	12	17
Spider Angiomata	30	20	20	29	39	20	34	26	27	33
Weight on admission	151	149	149	151	152	152	151	153	151	153
Mean gain in pounds										
First week	4	2	4	3	1	2	3	0	2	1
Second week	4	1	2	3	2	1	2	1	1	2
Third week	1	2	2	1	1	1	1	2	2	1
Total pounds gained	9	5	8	7	4	4	6	3	5	4

Table XV, Appendix II

The effects of two weeks of increasing ambulation on liver function tests and liver size and tenderness of the strict bed rest patients compared with the equivalent period in the course of the patients who were ambulatory throughout

	Strict Bed Rest	Ad lib Rest	P
I Liver Function Tests			
Total Serum Bilirubin			
Mean convalescent value ± 1 S. D.	1.01 ± .25	1.02 ± .26	
Mean change in first week	+.001	+.01	.8
Mean change in second week	-.12	-.09	.4
Prompt Direct Serum Bilirubin			
Mean convalescent value ± 1 S. D.	0.36 ± .12	0.38 ± .11	
Mean change in first week	-.021	-.025	.8
Mean change in second week	-.11	-.11	—
Bromsulphalein Retention			
Mean convalescent value ± 1 S. D.	3 ± 2.0	3 ± 2.0	
Mean change in first week	-.03	-.4	.1
Mean change in second week	-.7	-.8	.7
Total Patients	128	129	
II Clinical Findings - % of patients			
Return of minor symptoms	6	8	.5
Increase in size of liver by 2 cm. or more	5	3	.5
Appearance of liver tenderness	10	7	.5
Bromsulphalein "relapses"	6	2	.1
Total patients	126	127	

Table XVI

Total and prompt direct serum bilirubins on discharge to the Physical Reconditioning Center: comparison of the normal series with the first study patients and with the early and late groups in the third study

	Number	Range	Mean	S.D.	S.E.
Normal Series					
TSB	279	0.15 to 2.55	0.64	0.26	.028
PDSB	279	0.03 to 0.31	0.12	0.05	.005
First Study					
TSB	189	0.35 to 1.52	0.78	0.26	.019
PDSB	189	0.06 to 0.36	0.19	0.07	.005
Third Study - early exercise group					
TSB	88	0.33 to 1.48	1.00	0.23	.024
PDSB	88	0.14 to 0.71	0.40	0.11	.012
Third Study - Late exercise group					
TSB	86	0.30 to 1.40	0.89	0.23	.025
PDSB	86	0.11 to 0.49	0.30	0.10	.011

TABLE XVII, APPENDIX II

Comparison of follow-up sample with the remainder of the study patients with respect to mean values for age on admission, initial total serum bilirubin and duration of acute illness from admission to convalescence; comparisons of treatment groups in the sample according to the mean points (abnormality score) at discharge to duty and the interval from discharge to follow-up examination

Treatment group*	Number in sample	Mean age *yrs.*		Mean TSB *mg./100 ml.*		Mean duration *days*		Mean points for sample at discharge to duty	Mean interval *mos.*
		Sample	Rem.	Sample	Rem.	Sample	Rem.		
First study:									
DR	25	23	22	9.0	7.9	33	29	2	12
Dr	25	23	24	7.0	7.7	29	37	1	13
dR	25	25†	21	9.4	7.6	29	23	1	12
dr	25	24	23	8.9	7.5	29	25	1	12
Second study:									
CPS	11	23	24	7.1	9.8	23	31	2	8
CPs	11	22	22	9.4	7.2	31	25	1	9
cPS	11	26	23	10.0	8.4	30	30	2	8
cPs	11	22	23	6.3	9.4	29	34	1	9
CpS	11	24	24	7.4	7.3	33	29	1	8
Cps	11	27†	22	13.5†	7.9	49†	30	1	8
cpS	11	23	24	8.8	6.2	36	34	1	8
cps	11	23	24	7.2	7.6	27	36	1	10
Third study:									
E	44	24	23	10.3†	7.8	37	31	1	9
e	44	24	23	7.9	8.0	28	29	2	8

* See footnote to Table VII for explanation of abbreviations.
In addition, E = "early" and e = "late" exercise groups.
† Difference between the sample and remainder is significant at the 5 per cent level.

TABLE XVIII, APPENDIX II

Patients hospitalized in the interval between discharge and follow-up examination

Patients hospitalized for more than one day for:	First study				Second study							
	Diet		Rest		Protein		Calories		Supplements		Exercise	
	Forced	*Ad lib.*	Forced	*Ad lib.*	High	Normal	High	Normal	Added	Placebos	Early	Late
Complaints possibly related to hepatitis	5	3	5	3	1	0	1	0	1	0	1	0
Complaints probably not related to hepatitis	3	3	1	5	3	1	4	0	1	3	3	1
Complaints not related to hepatitis	6	4	7	3	4	5	3	6	4	5	8	1
Total patients hospitalized	14	10	13	11	8	6	8	6	6	8	12	2
Total patients followed	49	46	46	49	40	41	39	42	40	41	40	41

TABLE XIX, APPENDIX II

Results of follow-up study: Distribution of patients according to treatment group and abnormalities in symptoms, signs, and laboratory tests

		First study					Second and third studies									Both studies total	%
		DR	dR	Dr	dr	Total	Cp	CP	cp	cP	S	s	E	e	Total		
Total patients examined		24	24	25	24	97	21	21	22	22	42	44	43	43	86	183	
Abnormalities on follow-up	Points																
Mild–moderate symptoms	1	4	5	7	5	21	1	7	1	1	5	5	7	3	10	31	17
Severe symptoms	2	3	2	2	4	11	0	1	1	0	2	0	1	1	2	11	6
Hepatomegaly 3–5 cm.	1	2	2	2	3	9	1	6	6	3	10	6	9	7	16	25	14
Hepatomegaly >5 cm.	2	1	1	0	1	3	0	1	0	1	1	1	1	1	2	5	3
Liver tenderness	1	2	4	4	3	13	1	3	2	2	4	4	8	0	8	21	11
More than one spider	1	0	0	1	1	2	0	2	0	1	2	1	1	2	3	5	3
Splenomegaly	1	1	0	0	0	1	0	1	0	0	1	0	0	1	1	2	1
Total serum bilirubin	2	0	0	2	1	3	0	1	0	2	3	0	0	3	3	6	3
Prompt direct serum bilirubin	2	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1
Bromsulphalein test*	2	1	0/22	1	3	5	0/20	2/19	1	2/21	2	3	3	2	5	9	5
Cephalin flocculation	1	0	0	1	0	1	1	0	1	1	1	2	0	3	3	4	2
Thymol turbidity	1	0	1	2	2	5	1	1	1	1	2	2	2	2	4	9	5
Thymol flocculation	1	0	0	0	1	1	1	0	0	1	1	1	2	0	2	3	2
Total abnormality points																	
0		15	13	11	10	49	18	6	10	11	19	26	22	23	45	94	53
1		4	4	7	7	22	1	5	7	2	10	5	7	8	15	37	21
2		2	2	3	3	10	0	4	4	7	7	8	7	8	15	25	14
3		2	2	3	0	7	0	2	0	0	1	1	1	1	2	9	5
4		0	1	0	2	3	0	0	0	1	1	0	1	0	1	4	2
5		1	0	1	0	2	1	1	0	0	1	1	1	1	2	4	2
6		0	0	0	2	2	0	1	0	1	0	1	0	1	1	3	2
Total patients with complete data		24	22	25	24	95	20	19	21	21	40	41	40	41	81	176	

* The second number indicates the number of patients who had the test performed. In all other instances the total number is the same as at the top of the column.

TABLE XX, APPENDIX II

Characteristics of epidemics of infectious hepatitis among military personnel in various locales

Location	Korea and Japan	Germany (3)	United States (2)	Great Britain (34)	Italy (1) (36)	Middle East (35)
Dates	1951–52	1947–49	1944–45	1943–45	1943–44	1942–44
Number of patients	454	3,614	200	200	101 and 1,172	200
Per cent incidence of:						
Anorexia	95	Only 20% had acute onset	92	94	87	82
Malaise	92		68	58		82
Nausea	89		79	78	84	75
Liver pain	75		57	36		
Vomiting	64	Rare	58	50	51	33
Fever	53	Rare	42		61	53
Diarrhea	20		10	11	33	9
Per cent of patients with no symptoms prior to onset of jaundice	3 or 27 (a)	? High	10			16.5
Mean total duration of jaundice*—days	32	37 (d)		19	14–28	27
Mean duration of hospitalization—days	56 (b)	60	ca. 35	33	50–60	30
Per cent incidence of acute relapses						
Total	5.6	0.7 (c)	18.5	11.5	10–15	1.5
With jaundice	0	?	"Few"	ca. 7.5	5	
Mortality	0 in 454, (.2% in 2,448)	.2%	0	0	.2–.4%	0

(a) First figure—no symptoms prior to onset of clinical jaundice.
Second figure—no symptoms prior to onset of dark urine.
(b) First study only.
(c) Listed as per cent with "moderately severe" relapses.
(d) Estimated from data supplied by Dr. W. P. Havens, Jr. in which the duration from admission to a bilirubin of 1.5 mg./100 ml. was 27 days, and the duration of symptoms before admission 14 days.
* Since different criteria for the duration of jaundice were used in each study, these figures are approximations.

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